ARCHIVES OF PATHOLOGY

VOLUME 42 AUGUST 1946 NUMBER 2

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RADIATE FORMATION ON PATHOGENIC FUNGI IN HUMAN TISSUE

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THE APPEARANCE and the nature of pathogenic fungi seen in tissue have been for the most part well described. Of particular interest is the finding, on pathogenic fungi in tissue, of the peculiar phenomenon known as radiate formation. This appearance of radiation has come to be accepted as a commonplace with various organisms, notably species of Actinomyces or Nocardia (ray formation) and Coccidioides immitis (prickles). Such radiate formation is quite commonly encountered in experimental aspergillosis (Aspergillus fumigatus) in laboratory animals but is still a rarity in man. In certain cases of sporotrichosis a radiating substance has been seen in tissue of man—but only in a very few instances. Why these formations occur has never been satisfactorily explained. The views and theories advanced have been many and diversified. Attention was drawn to this structure recently when radiate formation was observed on cells of Sporotrichum schencki in tissue[1] and on cells and filaments in 2 human cases of aspergillosis. As a result, an attempt is being made to determine just why such structures are formed and under what conditions. This paper will be devoted to the presentation of such organisms with radiate formation as have been found in human tissue, the histopathologic picture within which such structures are seen and the prevailing theories which have been advanced in the effort to explain the phenomenon.

ACTINOMYCOSIS

Definition.—Actinomycosis is a local or systemic disease, granulomatous in nature, and may be acute, subacute or chronic. It is characterized chiefly by sinuses and fistulas from which may be isolated variously colored granules, which are masses of mycelium of species of

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1. Moore, M., and Ackerman, L. V.: Arch. Dermat. & Syph. **53**:253, 1946.

the genus Actinomyces (Nocardia, Discomyces, Streptothrix, Actinobacillus, Cohnistreptothrix, Brevistreptothrix and Proactinomyces).

History.—The disease as it occurs in man is usually considered to have been first described by Israel [2] in 1878, when he observed the radiating fungus in the pus of a patient suffering with empyema. This followed what was considered the original observation of the infectious process known as "lumpy jaw" in cattle and described by Bollinger [3] in 1877. The organism in cattle was described and named by Harz (in Bollinger's report) Actinomyces bovis. In 1885 Murphy [4] reported the disease in America for the first time, three years after Ponfick [5] had shown the similarity of the infections in man and animal.

Negroni and Bonfiglioli [6] have credited Lebert, of Paris, France, with having been the first to observe this disease in man, in 1848. Israel in his publication of 1878, however, reported that he showed his preparation to Professor von Langenbeck, who recalled a case seen by him in 1845 at Kiel, Germany. Israel included in his paper a résumé of the case described by von Langenbeck. His figure 9 c shows the characteristic radiate formation of Actinomyces which von Langenbeck described as *cylindrische Körperchen von eigenthümlicher Lichtbrechung* (cylindric bodies of a peculiar refraction of light). It is apparent, therefore, that credit should be given to von Langenbeck for the observation of the first human case of actinomycosis showing radiate formation.

Pathology.—Grossly, actinomycosis of the skin manifests itself in the form of nodules which ulcerate to produce suppuration and sinus formation and which may result in scar tissue. The sinuses become intercommunicating to produce eventually a large tumefaction with granulation tissue and multiple sinuses exuding seropurulent or serosanguineous material onto the skin surface. In the internal organs, the nodules rupture; the pus is spread through the tissue, attacking bones as well as soft tissue, and abscesses develop which eventually form the sinuses described.

Microscopically, the lesions of actinomycosis are granulomatous in nature. At the early stage a small nodule develops, with the organism in the center of the node. The area is infiltrated by lymphocytes, polymorphonuclear leukocytes, eosinophils and large, irregular macrophages. This area in turn is surrounded by plasma cells and proliferating con-

2. Israel, J.: Virchows Arch. f. path. Anat. **74**:15, 1878.

3. Bollinger, O.: Centralbl. f. d. med. Wissensch. **15**:481, 1877.

4. Murphy, J. B.: New York M. J. **41**:17, 1885.

5. Ponfick, E.: Die Actinomykose des Menschen, eine neue Infectionskrankheit auf vergleichend-pathologischer und experimenteller Grundlage geschildert, Berlin, A. Hirschwald, 1882, p. 138.

6. Negroni, P., and Bonfiglioli, H.: J. Trop. Med. & Hyg. **40**:206 and 240, 1937; Rev. Soc. argent. de cien. nat. **15**:159, 1939.

nective tissue cells. The connective tissue surrounding the whole area becomes noticeably edematous and is infiltrated by polymorphonuclear leukocytes and lymphocytes. As the organism grows, the bacillary forms develop filaments, which become intertwined. The cells around the fungous elements show degenerative changes and are finally replaced by invading leukocytes. The result is a central area of fungous elements which are intertwined and compact and which may show either radiate or "club" formation or simply a mass of filaments. This is the granule seen in smears of pus. The granule is surrounded by various leukocytes, many showing degenerative changes and cellular debris. The edematous connective tissue surrounding this area appears as granulation tissue. Macrophages, many showing phagocytosed fat, can be seen in the neighborhood; and some of these large cells may invade the pyogenic mass. The nodules may remain discrete, or they may coalesce to form large masses. As this large necrotic area increases in size and forms a frank abscess, the pus seeks more space and consequently burrows through the adjacent tissue. The path created by the pus constitutes a sinus. The sinuses do not heal easily because of the constant flow of pus, with the result that numerous such sinuses may be formed, many becoming intercommunicating. Large pockets of pus may develop along the path of the sinus. The sinus eventually becomes filled in with granulation tissue, which may show scattered leukocytes and numerous newly formed blood vessels and young connective tissue cells.

Fungus in Tissue.—The organisms of actinomycosis may be divided into two main groups, the aerobic and the anaerobic or microaerophilic. The aerobic forms, which some workers regard as a distinct biologic group and therefore classify as Nocardia, exemplified by Nocardia asteroides, is made up of both pathogenic and saprophytic organisms, some having acid fastness as a property. The second group, the anaerobic or microaerophillic forms of Actinomyces, is considered by some as being made up of a single species, Actinomyces bovis, or, as preferred by Dodge[7] and also by Negroni, Actinomyces israeli. This is the pathogenic actinomycete commonly encountered in human tissue, which is gram postive and non-acid-fast and is chiefly responsible for the so-called sulfur granules.

In spite of the controversy over the classification of Actinomyces, in tissue the fungus in its late stage may be seen as a granule, which may be white, whitish yellow, black, green or red and may or may not show the clubbed or radiate formation (fig. 1, *1* and *2*). When Actinomyces first invades human tissue, it does so either in the form of a bacillus-like cell, which is approximately 0.2 to 0.6 micron in diameter and somewhat irregular in form, or as nonseptate, occasionally branching filaments, which vary in length but are approximately

7. Dodge, C. W.: Medical Mycology: Fungous Diseases of Man and Other Mammals, St. Louis, C. V. Mosby Company, 1935, p. 714.

15 microns. The fungus in the early form is carried through either the blood or the lymph stream and comes to rest at some locus in which, if the conditions are favorable to it, the organism grows and multiplies.

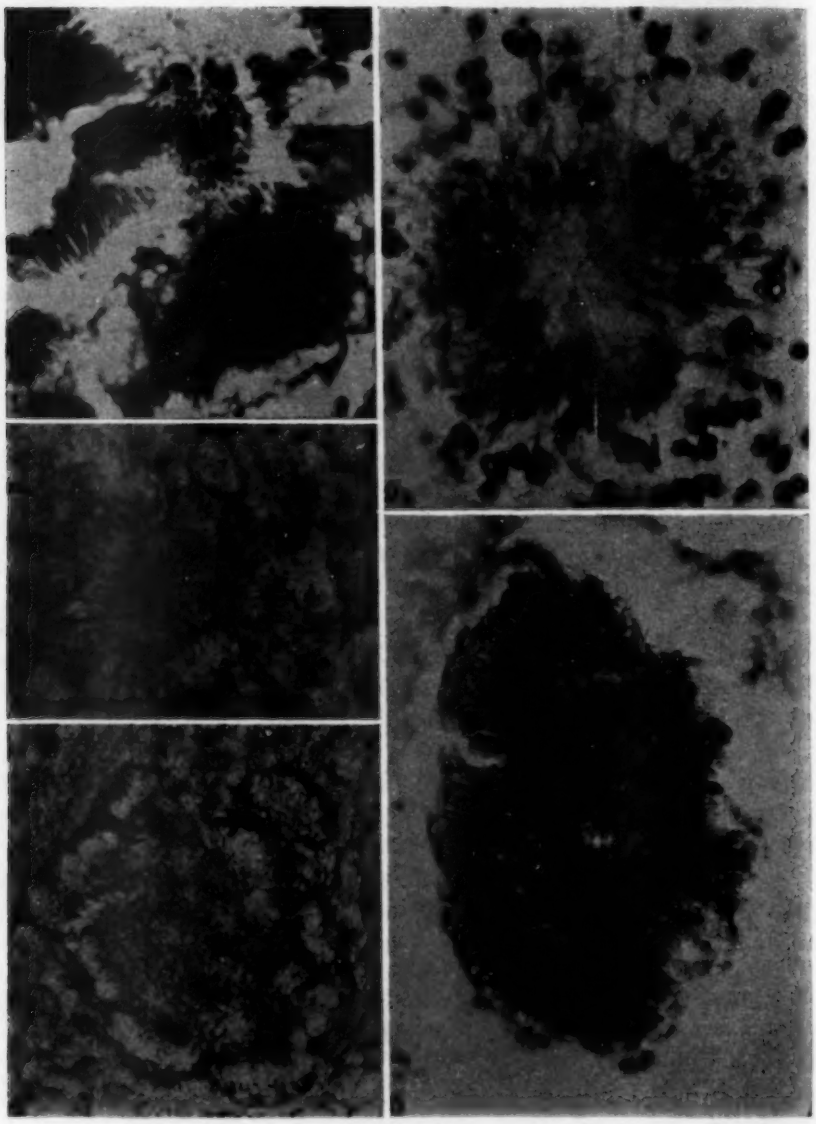

Fig. 1.—*1.* Radiate formation of actinomycotic granules; hematoxylin and eosin; × 558. *2.* Actinomycotic granule minus clubs in the brain; hematoxylin and eosin; × 988. *3.* Soft radiating granule of Actinomyces in pus; × 1052. *4.* Hard granule of Actinomyces showing lobulate arrangement, seen in pus; × 574. *5.* Actinomycotic granule showing organisms and radiate formation, seen in skin: Gram-Weigert stain; × 446.

The bacillary form or the filaments elongate, branch and become intertwined and compact to make up the young granule. The growth process continues and, as a result, there develops one of two types of granules, the soft or the hard. The soft granule may show in its center a few degenerate polymorphonuclear leukocytes or leukocytic granules. The granule proper consists of filaments which may be intertwined but have peripheral extensions (fig. 1, *3*). The young hard granule when freshly isolated is small and has a central region consisting of leukocytes or leukocytic granules and intertwined filaments. Extending from the periphery in the form of rays, and usually covering the whole granule, is a hyaloid or gelatinous-like substance. At the periphery of the granule these extensions appear to engulf the filaments of the fungus, with the terminal portions appearing filiform, flattened or, usually, swollen or club shaped. When the granule is older and when placed on a glass slide, it is usually resistant to crushing, which often causes the cover slip to be broken. These older granules appear to have the consistency of small calcified nodules. When examined microscopically, they are seen to consist of lobulated masses (fig. 1, *4*). The central area is amorphous in nature, while the lobulated periphery is hard and glasslike in appearance, refractile and made up of radiating rays or clubs. Some of the radiations may appear either as flattened, broad or as needle-like projections, with a yellowish tinge, and seem very refractile. When stained with hematoxylin and eosin, the center of the granule shows leukocytes or leukocytic and pigmentary granules, which stain heavily. Surrounding this is the amorphous-like material consisting of filaments and some degenerate cells, which may also stain deeply with the hematoxylin. The periphery of the granule, consisting of the radiations or clubs, takes the eosin stain and is said to be acidophilic. These peripheral extensions were thought to be merely the growing, swollen tips of the fungus, since in some cases they appeared to be closely adherent to the filament. When stained by the Gram-Weigert method, however, the filaments of the fungus are gram positive, while the clubs take the eosin stain, showing that the two are distinct entities (fig. 1, *5*).

ASPERGILLOSIS

Definition.—Aspergillosis as a disease of man is caused by species of Aspergillus with the production of chronic inflammatory and granulomatous lesions of the pulmonary system, the external auditory canal, the mucous membrane of the conjunctiva, the cornea, the nasal sinuses, the kidney and occasionally of the skin, the nails, the bones and the meninges.

History.—The first description of a human case of mycosis which may be doubtfully considered as one of aspergillosis is that attributed to

Bennett, who in 1842 found fungi in the sputum of a patient. Rénon [8] contributed a comprehensive review of the early literature with a study of aspergillosis in 1897, and he credited Sluyter with being, in 1847, the first to describe a case of pneumonomycosis actually caused by Aspergillus. The patient was a woman who had died of a pulmonary infection. In a cavity of the lung, Baum, Litzmann and Eichstedt found black, adherent masses made up of mycelial filaments and round bodies. Some of the filaments emerging from the mass had swollen extremities on which these observers noted a large number of ovoid cells. They considered the organism a Mucor, but Virchow, who studied the material in detail, believed that it was probably an Aspergillus.

The first scientific report of aspergillosis was made by Virchow [9] in 1856, when he reported 4 cases of bronchomycosis and pneumonomycosis due to Aspergillus. The 4 patients had died, respectively, of dysentery, pulmonary inflammation with emphysema, carcinoma of the stomach and pneumonia. Reports of a number of other cases followed, and these have been reviewed by Rénon (1897), by Lang and Grubauer [10] (1923) and by others.

The first observation of the radiate or actinomycetoid form of Aspergillus in tissue was made by Lichtheim [11] in 1882 when he injected Aspergillus fumigatus, obtained from the lung of a woman at necropsy, into a rabbit. In 1884 Laulanié [12] observed the radiating forms in the lung of a rabbit and described them as having the appearance of a rosette, with the very short extensions yellow, refractile, swollen at the free end and needle shaped at the adherent end. These forms have since been observed in numerous animals by many investigators, both in spontaneous and in experimentally induced aspergillosis.

The radiating form of Aspergillus was described as it occurs in man, perhaps for the first time, by Wheaton [13] in 1890. He observed a case of pneumonomycosis involving not only the lungs and the bronchi but also the lymph nodes. The patient was a 2½ year old girl who had a slight cough and showed a loss of weight for two months. Wheaton found small cavities filled with pus, and in the surrounding tissue there were bright orange-colored bodies the size of mustard seeds. The rosette-like bodies seen in the lung, as illustrated in the accompanying

8. Rénon, L.: Etude sur l'aspergillose chez les animaux et chez l'homme, Paris, Masson & Cie, 1897, p. 300.

9. Virchow, R.: Virchows Arch. f. path. Anat. **9**:557, 1856.

10. Lang, F. J., and Grubauer, F.: Virchows Arch. f. path. Anat. **245**:480, 1923.

11. Lichtheim, L.: Berl. klin. Wchnschr. **19**:129 and 147, 1882.

12. Laulanié, F.: Arch. de physiol. norm. et path. **4**:487, 1884.

13. Wheaton, S. W.: Tr. Path. Soc. London **41**:34, 1890.

woodcut, were the characteristic radiating structures. A fumigatus was obtained in culture. In 1893 there appeared two reports on the actinomycetoid form of Aspergillus in human tissue. One, by Boyce,[14] described macrophages of varying size which engulfed masses of hyphae. In describing the structures, he pointed out that "stains have very little effect upon them, the most powerful nuclear dyes rendering the nuclei only just visible; the cell substance has a very characteristic yellow tinge, and typical amoeboid outline; the pseudopodia may be long and blunt, and often have a ground-glass appearance, which contrasts with the clearer and more granular condition of the rest of the cell." His illustration of a nodule in the lung showed little, if any, of the hyaloid, ground-glass-appearing substance but showed mostly radiating filaments of the fungus, such as one sees in culture. However, other illustrations depict brownish encrusting substances on the filaments, some in the form of swellings and others looking as if they were macrophages, which were similar to some seen in a case which will be described. The other paper published in 1893, by Kohn,[15] described granules in lung tissue in a case of aspergillar pneumonomycosis which were similar to those of actinomycosis and from all sides of which long slender filaments extended outward.

The occurrence of Aspergillus as an agent of mycetoma has been recognized in the literature. The occurrence of the organism in this disease, however, is rare. In 1930 da Fonseca[16] reported a case of Madura foot caused by Aspergillus amstelodami. Biopsy of the tissue revealed numerous hard, rounded granules, many having their surfaces covered with extensions. The granules were sulfur yellow to green. His microscopic observations of the granules and the accompanying illustrations are in keeping with the characteristic radiating aspergillotic granules.

Two unreported cases of aspergillosis of the lungs with radiate formation have been seen and diagnosed as such by me. The first case was that of a patient at the United States Marine Hospital at Staten Island, N. Y. Slides of tissue made from a localized lesion of the lung were received in 1942 from Dr. J. A. Pasternack, at that time director of pathology. The sections consisted almost entirely of fungous elements, with little tissue. In one area of the section there were masses of filaments which seemed to be undergoing hyalinization, leading to the ground-glass-like appearance. These masses took the eosin stain except for some areas which did not become colored but

14. Boyce, R.: J. Path. & Bact. **1**:163, 1893.

15. Kohn, H.: Deutsche med. Wchnschr. **19**:1332, 1893.

16. da Fonseca, O., Jr.: Rev. med.-cir. do Brasil **38**:415, 1930.

appeared yellowish and refractile. In this zone were seen the typical radiating structures of Aspergillus.

The second case was that of a 29 year old man seen on the chest service of Dr. Evarts Graham at Barnes Hospital, St. Louis. The patient had noted recurrent hemoptysis for the past fifteen years, which became worse on exertion. A roentgenographic study disclosed an infiltration of the middle lobe of the right lung, and a bronchogram showed an obstruction in the right middle lobe bronchus. Bronchoscopy revealed partial stenosis of the right middle lobe bronchus and a small amount of blood and pus coming from the orifice of this lobe. The right lung was removed. An area of definitely diseased lung was found in the region usually occupied by the middle lobe, but actually it was part of the upper lobe of the right lung. It consisted of contracted scar tissue with some surrounding atelectasis and inflammatory infiltration. Five millimeters beneath the area of adhesions and fibrin there was a thick-walled abscess cavity, which was diagnosed as a chronic abscess of the lung. The cavity was 12 mm. in diameter, with a hard, white, resilient wall. The inner wall of the cavity was covered with soft, friable, brown material. The parenchyma distal to the cavity in the upper lobe showed fibrosis and hemorrhage as well as dilatation of the bronchi. On microscopic examination, masses of fungi and inflammatory cells were seen in the cavity and in the dilated bronchi. In the surrounding parenchyma there was considerable fibrosis, as well as a giant cell reaction, many of the giant cells phagocytosing fungous filaments. The diagnosis of chronic abscess of the lung and aspergillosis was made.

Pathology.—Aspergillosis is usually characterized by the presence of edema and erythema. The inflammation and the inflammatory response may be so pronounced as to bring about very noticeable changes in the affected and the surrounding tissue. Pulmonary involvement is perhaps the most common of the infectious processes. In the lungs the lesions are chiefly of the inflammatory or tubercle-like types. Small white to yellowish white nodules may be seen grossly in the involved areas. The involvement may be parenchymatous or interstitial. In parenchymatous aspergillosis the mucosa of the bronchi when involved may show congestion with or without ulceration and membranous patches. Lesions of the alveolar sacs may remain localized with the formation of tubercle-like structures and abscess-like accumulations of cells or they may become widespread and generalized. The abscesses are usually seen in the smaller bronchi, from which they may push through to involve the lung tissue. The pleural surface may become infected, and inflammation, congestion, thickening and fibrosis follow, with resultant pleuritic pain.

Interstitial aspergillosis usually does not remain localized; it tends to involve the alveoli, reproducing the pathologic processes noted in the foregoing paragraph. Thrombi may be formed in the infected arterial lumens, and atheromatous patches may be seen throughout the arterial tree, in bronchopulmonary involvement.

Microscopically, the cellular infiltrate is similar in many respects to that seen in other mycotic granulomas. The infiltrate may be diffuse, or it may be circumscribed, as in the case of localized, nodular lesions. Interspersed among or surrounding the mass of fungous filaments may be seen leukocytes, leukocytic granules, lymphocytes, plasma cells and giant cells of the Langhans type. Central necrosis may or may not be present, while the whole response may be somewhat encapsulated with fibrous material. The giant cells may be few or many and often are arranged in tubercle-like fashion. Many of the giant cells engulf fungous elements. In some of the giant cells can be seen yellowish, nonstaining, refractile elements, which appear to be not the fungous filaments described but the hyaloid, encrusting or radiating substance seen in the radiating granules.

Fungus in Tissue.—Aspergillus assumes various forms in tissue and thereby differs greatly from some of the common pathogenic fungi. In culture the fungus is characterized by the production of filaments, spores of various types and spore heads or conidiophores with the development of conidia. Except in aural infections and perhaps experimentally produced disease, the conidiophores are not often seen in human tissue. The ear, with its moistness, warmth and excreta, serves as an excellent culture medium for the growth of fungi and may well be compared with a test tube or a culture plate. The conidiophores grow abundantly and luxuriously on the surface. The penetrating organism, however, consists chiefly of filaments and large round or ovoid spores, perhaps comparable to the chlamydospores seen in cultures. In organs in which there is not the amount of free air present that one finds in the ear, the fungus is seen to consist chiefly of branching, interlacing, septate hyphae or filaments. In the lung, where the organism may be found in large masses either free in the tissue or in bronchi or bronchioles, there can be seen layers of large spherical to ovoid cells adjacent to the peripheral filaments of the growing mass. These are the so-called chlamydospores.

The radiate structure on Aspergillus in tissue also assumes various forms. It may be seen on a single, thick-walled (double-contoured), spherical cell or spore, on a short or a multicelled filament or as extensions on a granule made up of a mass of filaments and spores. The simplest form shows a central thick-walled spore with peripheral extensions (fig. 2, *1*). These radiations may be somewhat uniform in

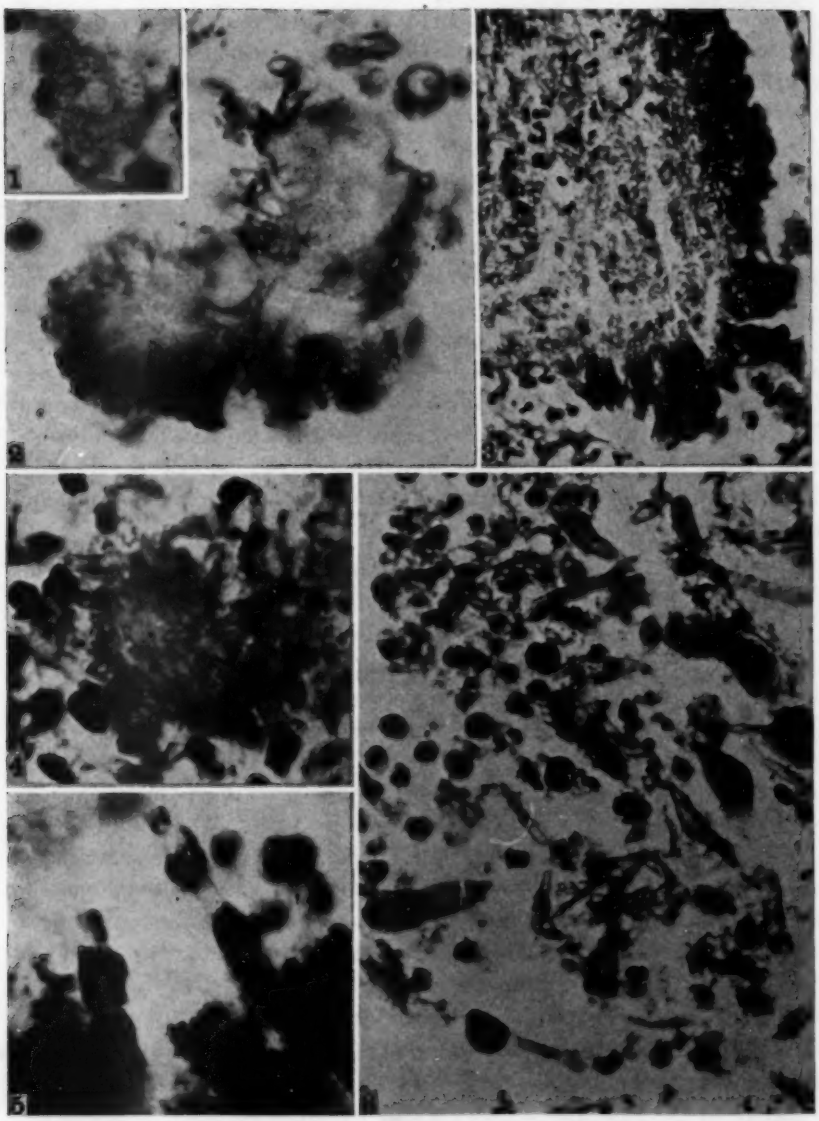

Fig. 2.—*1.* Radiate formation on a single cell of Aspergillus; hematoxylin and eosin; × 1,110. *2.* Radiate formation on a branching hypha of Aspergillus; hematoxylin and eosin; × 1,110. *3.* Periphery of aspergillotic granule showing acidophilic material, seen in lung; hematoxylin and eosin; × 350.5. *4.* Aspergillotic granule in lung, showing ground-glass-like appearance with radiating crystalline-like substance; hematoxylin and eosin; × 853. *5.* Incrustations on hyphae of Aspergillus; hematoxylin and eosin; × 1,222. *6.* Incrustations on cells and filaments of Aspergillus; hematoxylin and eosin; × 510.

size and shape, or they may be irregular; at any rate they suggest the asteroid type described by others. The rays are hyaloid to ground-glass-like in appearance; they may stain with eosin, or they may remain unstained, in which case they are yellowish to light yellowish green (fig. 2, *2*). The tips of the rays are not swollen or rounded, as in the club formation seen in actinomycotic rays, but appear more as elongated crystals with "broken-off," or abrupt tips. In some cases the radiations appear more as a uniform crystalline mass with short angular projections or as segmented forms. In some cases the radiations appear completely around the central fungous cell, giving the whole the appearance of a spiny ball (fig. 2, *4*). The radiations on the filaments are somewhat similar in that they appear along the length of the filament in much the same fashion as around the spherical cell. They usually are of unequal length, appear at times like crystalline needles, although somewhat greater in diameter, but in general conform to the tips already described.

The granules when seen in tissue vary somewhat in size but usually are 1.5 mm. in diameter; they are hard, yellow to yellowish green and not lobulated, as are the actinomycotic granules. Radiations may or may not be present, but usually are rare. The nonradiating granule is a compact mass of intertwining septate filaments. Toward the periphery of the granule can be seen large spherical to ovoid cells, the chlamydospores. These cells may be seen at times in the form of a layer. The periphery of the granule is made up of loosely interwoven hyphae, scattered among which may be seen many leukocytes, chiefly polymorphonuclears, and other inflammatory cells. The granules with peripheral extensions or radiations when seen in cut sections stained with hematoxylin and eosin are composed of three zones. The central area is made up of compactly interwoven filaments which stain lightly. Surrounding this is a zone which stains more intensely with hematoxylin and also shows a substance which appears to be in the form of an incrustation on the hyphae and which stains deeply as if with a mixture of hematoxylin and eosin. This zone is more or less amorphous in appearance and apparently is fairly brittle, since one sees cracking, which is absent in the central zone. When this area is examined under higher power, filaments and chlamydospores can be discerned within it. Surrounding the midzone is an area of loosely interwoven hyphae with many of the large spherical to ovoid cells capped by the eosinophilic or acidophilic staining, irregular radiations or ray formations (fig. 2, *3*). These may or may not surround the entire granule. Usually, however, in cut sections they may be seen involving only part of the periphery, with the uninvolved areas consisting of masses of filaments engulfed by inflammatory types of cells as noted in the

nonradiation granules. One may also see at various points within the granules areas made up entirely of the eosinophilic substance, whereas furrows or spaces may be seen which are devoid of fungi, being filled instead with leukocytes.

In addition to the radiating types described, there is another type of formation which has been observed in 1 of the cases noted and which Boyce described and illustrated in his paper as a macrophage (see his fig. 6). These "macrophages" stain with eosin, or they may remain unstained, and have a yellowish color and appear to invest or engulf a whole or a part of a hypha or groups of hyphae; or they may appear as one to several nodules on a hypha. Their appearance is more that of an incrustation or concretion than that of a macrophage (fig. 2, *5* and *6*). On closer examination, however, it is apparent that the engulfing substance is similar to, if not identical with, that of the radiating forms seen on other filaments and granules. In 1925 Dillard and Weidman [17] described 2 cases of multiple hemorrhagic sarcoma of Kaposi. In the second case the patient, an 82 year old laborer, was admitted to the hospital with bronchopneumonia. He died twenty-four hours after admission, and necropsy was performed. When the gastrohepatic lymph node was being examined, there were seen filaments or mycelia, both free and in giant cells (see their figs. 13 and 14), which were described as "homogeneous, highly refractile and hyaloid." It is apparent from the description of both the fungi and the histopathologic appearance of the lesion that the fungus was Aspergillus. In their figure 14 are illustrated fungous cells with thick coatings and "chlamydospores" which certainly correspond to the radiating forms described in this paper. It is probable that the forms described by Weidman and Douglas [18] in a paper published in 1921 may likewise have been such radiating forms of Aspergillus (see their fig. 10). In 1932 Weidman [19] compared the "chlamydospores" seen in the case published in 1925 with the Hülle cell of Aspergillus, as described and illustrated by Thom and Church, who refer to the work of Eidam. As Weidman correctly put it, the Hülle cell is a thick-walled cell of an aborted reproductive structure which actually is comparable with the true chlamydospores formed under adverse conditions of growth. This being the case, it is doubtful whether the structures seen by Wiedman could be Hülle cells, since reproductive structures in parasitized tissue are not the rule and, further, the engulfing substance was found on filaments as well, which certainly could not be interpreted as fruiting bodies.

17. Dillard, G. J., and Weidman, F. D.: Arch. Dermat. & Syph. **11**:202, 1925.
18. Weidman, F. D., and Douglas, H. R.: Arch. Dermat. & Syph. **3**:743, 1921.
19. Weidman, F. D.: Arch. Path. **13**:725, 1932.

COCCIDIOIDOMYCOSIS

Definition.—Coccidioidomycosis is a mycotic infection caused by C. immitis, which may manifest itself in two chief forms: (*a*) a respiratory disease, which may be acute, self limited in duration and benign; and (*b*) a chronic, progressive granulomatous form—coccidioidal granuloma—with remissions, relapses and widespread dissemination (systemic), involving skin and visceral and osseous structures.

History.—The disease was first described in 1892 by Posadas [20] from Buenos Aires, Argentina, as a new case of mycosis fungoides. In the same year, Wernicke [21] reported this case in another journal. Two years later, the second instance of the infection was reported in the United States by Rixford.[22] In 1896 Rixford and Gilchrist [23] published in detail the second and also the third case of the infection. The organism, at first considered to be protozoon, was named Coccidioides by Stiles, a medical zoologist. However, the microbe was found to be a fungus and now bears the name C. immitis. Up to the present time a large number of cases of the disease have been discovered, and these chiefly in such endemic regions as the San Joaquin Valley, Calif., and in parts of Texas, New Mexico and Arizona. In Argentina the incidence of the disease is still extremely low.

The first observation of radiate formation on cells of C. immitis in human tissue was made perhaps by Rixford and Gilchrist in 1896, when they figured a cell (see their fig. 17) with the description that the "parasite presents a number of fine prickles." These prickles extended out from the capsule. In 1926 Ahlfeldt [24] called attention to these radiations in experimentally produced disease in guinea pigs, stating that she found the prickles in several sections (see her fig. 1) and that they were found only in adult organisms that were ready to liberate young forms or the spores. In a later publication [25] there are drawings also of radiating forms of C. immitis. In 1932 de Almeida observed radiate forms of C. immitis in guinea pigs experimentally infected with this organism. These observations were made in 3 animals with three different strains of the fungus. Radiate formation was seen by de Almeida in 2 human cases of the disease. The radiation effect on cells of C. immitis in human tissue has since been noted by others.

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20. Posadas, A.: An. d. Circ. méd. argent. **15**:585, 1892.
21. Wernicke, R.: Centralbl. f. Bakt. (Abt. 1) **12**:859, 1892.
22. Rixford, E.: Occidental M. Times **8**:704, 1894.
23. Rixford, E., and Gilchrist, T. C.: Johns Hopkins Hosp. Rep. **1**:209, 1896.
24. Ahlfeldt, F. E.: Arch. Path. **2**:206, 1926.
25. Ahlfeldt, F. E.: J. Infect. Dis. **44**:277, 1929.

Pathology.—The reaction of the tissue to C. immitis in the progressive or granulomatous form of the disease is similar in many respects to that noted in other mycotic granulomas. Microscopically, the skin appears acanthotic, with pronounced parenchymatous or interstitial edema and vesicles in the epidermis. Abscesses may be seen throughout the region involved in the inflammatory process, but these do not, as a rule, reach the large proportions of those noted in blastomycosis. The nodular type of lesion usually suppurates and becomes necrotic, but definite large abscesses are rarely formed. Where the fungus is found, however, abscesses may be very apparent, as well as an inflammatory response. In the dermis there is a granulomatous response with an infiltration and proliferation of lymphocytes and of plasma, epithelioid and giant cells the last of the Langhans type. Newly formed blood vessels are apparent, and there is a tuberculoid response with the formation of tubercles which may show caseation and liquefaction necrosis followed by fibrosis and calcification. The edema in the epidermis may extend into the corium and the subcutis, while in other cutaneous lesions the hyperplastic epidermis may be accompanied by whorl and pearl formation not unlike that seen in a carcinomatous process.

In its systemic spread the organism may cause the formation of abscesses, areas of necrosis and granulation tissue. In the lungs the lesions may simulate grossly those of miliary bronchopneumonia or bronchiolitis, acute or chronic miliary tuberculosis, miliary carcinomatosis or secondary stage silicosis. Induration, cavitation and caseation may result. The disease is less marked in the region of the mediastinum than in the periphery of the lung field. Lesions are usually not present in the esophagus and the small intestine. The microscopic observations in the systemic spread are quite similar to those in the cutaneous involvement, with noticeable edema, infiltration and proliferation of the various cells already named, necrosis, tubercle formation, caseation, liquefaction and, finally, fibrosis and calcification.

Fungus in Tissue.—In tissue, pus, pleural fluid, sputum or exudates C. immitis is seen as a thick-walled spherical structure, measuring from approximately 2 to 80 microns in diameter, and may be either a simple, nonbudding cell or a large sac filled with endospores. In its host the fungus reproduces by endospore formation. The spores are set free by rupture of the wall of the mother cell. The spores enlarge; endospores are formed, and the process repeats itself. In the normal adult form the endospores are many. Occasionally, there may be large cells containing only a few spores, and they are generally considered to be immature.

Radiate formation on cells of C. immitis in tissue varies from small prickles, as described by Rixford and Gilchrist and by Ahlfeldt,

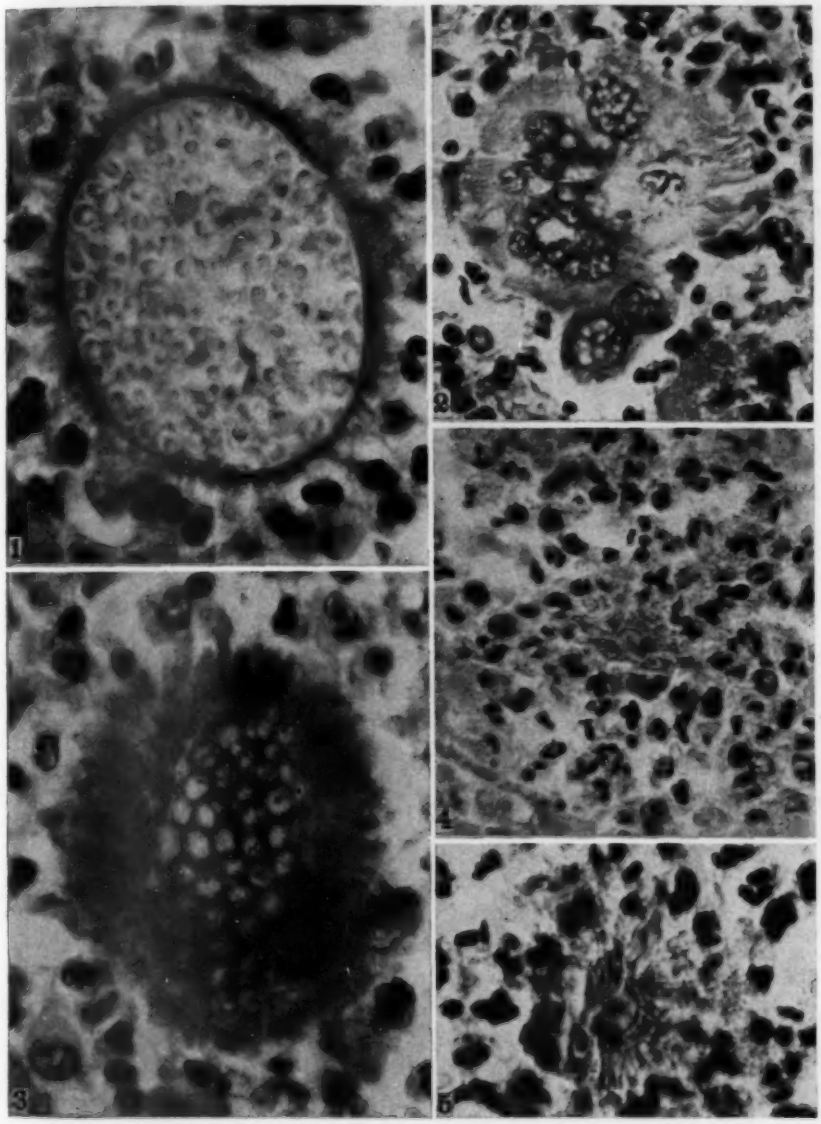

Fig. 3.—*1.* Endosporulating cell of Coccidioides immitis showing prickle formation; hematoxylin and eosin; × 1,110. *2.* Group of spores and mother cells of C. immitis with radiating acidophilic material; hematoxylin and eosin; × 558. *3.* Endosporulating cell of C. immitis showing club formation; hematoxylin and eosin; × 972. *4.* Radiation on spherical cell of Sporotrichum schencki; methylene blue and eosin; × 669. Note the leukocyte infiltrate. *5.* Radiations on a thick-walled spherical cell of S. schencki showing abrupt, "broken-off" tips of rays; methylene blue and eosin; × 1,110.

to large radiating forms. The prickles may be fine or broadened, measuring approximately 2 to 5 microns (fig. 3, *1*). Ray forms may be seen as elongated, ovoid structures, varying in length and simulating actinomycotic radiations, or they may be rather long tubular projections, such as those described by de Almeida (fig. 3, *3*). Occasionally, the eosinophilic radiations can be found extending from a group of spores of an apparently freshly ruptured mother cell, or they may appear to be engulfing groups of spores as well as immature mother cells (fig. 3, *2*). In all observations of radiating cells of C. immitis, as in the case of both Actinomyces and Aspergillus, the microscopic picture of the tissue response remains constant. These structures are seen in areas heavily invaded by leukocytes and by cells of an inflammatory type and are in close contact with these cells.

SPOROTRICHOSIS

Definition.—Sporotrichosis is a granulomatous process which is usually confined to the cutaneous or the subcutaneous tissue. The organisms, members of the genus Sporotrichum, may spread through the lymph channels to produce various lesions of the skin, or they may involve the internal viscera and bony structures.

History.—The first pathogenic species of Sporotrichum was probably that described by Montagne.[26] The organism was isolated from a case of bronchomycosis by Gübler and was named Sporotrichum bronchiale. The important knowledge of the disease, however, dates from the report of Schenck [27] in 1898 and the later one of Hektoen and Perkins,[28] who named their fungus S. schencki. Much of the present day knowledge of sporotrichosis is attributed to de Beurmann and Ramond,[29] of France, who in 1903 reported a case of sporotrichosis which was found by Matruchot and Ramond (1905) to be due to an organism which they named Sporotrichum beurmanni. In the following years de Beurmann and Gougerot and many other French workers established the identity of the disease in that country, while many reports appeared in the United States, Brazil, Argentina, Colombia, Germany, Austria, England, Turkey, Spain and Italy. These isolated reports were given greater attention when de Beurmann and Gougerot [30] published a collection of approximately 200 cases of sporotrichosis with a study of the disease and the organism.

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26. Montague, cited by Saccardo: Sylloge fungorum omnium huiusque cognitorum. Patavii, typis seminarii, 1886, vol. 4, p. 100.

27. Schenck, B. R.: Bull. Johns Hopkins Hosp. **9**:286, 1898.

28. Hektoen, L., and Perkins, C. F.: J. Exper. Med. **5**:77, 1900.

29. de Beurmann, L., and Ramond, L.: Ann. de dermat. et syph. **4**:678, 1903.

30. de Beurmann, L., and Gougerot, H.: Les sporotrichoses, Paris, Félix Alcan, 1912, p. 852.

The first observation of radiation on cells of Sporotrichum in human tissue was made by Splendore [31] in 1908, who termed the structure asteroid formation. The star-shaped bodies were found extracellularly in pus obtained from a verrucous, vegetative, hard and somewhat elastic lesion that had been present for twenty days on the right side of the face of an Italian woman living in São Paulo, Brazil.[32]

In 1908 Greco [33] described radiating forms in man and rat from Argentina. The following year, Harter and Gruyer,[34] in France, were able to demonstrate radiating forms of Sporotrichum in experimentally produced sporotrichosis in a guinea pig.

In 1934 Bordes, Berhouet and Errecart [35] published 4 cases of sporotrichosis from Uruguay. While examining hematoxylin and eosin–stained sections of a gummatous lesion in 1 of these cases, Talice noted the asteroid form described by Splendore. In 1935 Talice [36] published this case and an additional one in which radiating forms were shown. In 1939 MacKinnon [37] reported that he and Talice had found radiating sporotricha in the pus of 6 of 7 cases of sporotrichosis.

Moore and Ackerman [1] have recently observed the asteroid form of Splendore in sections of a lesion of gummatous sporotrichosis with lymphatic spread which occurred in a man aged 53 years, a native Missourian. This case is the first observed in the United States.

Pathology.—Sporotrichosis is characterized by the production of various types of cutaneous and systemic lesions. These can be listed in brief as gummas, ulcers, furuncle-like lesions, abscesses, nodules associated with lymphadenopathy and a general granulomatous response in skin, internal viscera or bony structures. The microscopic response varies according to the type of lesion present. In general, however, there is edema in the epidermal layers associated with irregular acanthosis, extensive or moderate, and at times suppuration, foci of which are particularly evident in the ulcerating type of lesion. There is a prominent infiltrate of polymorphonuclear leukocytes scattered throughout the pseudoepitheliomatous growth in the cutis, forming

31. Splendore, A.: Rev. Soc. de sc., São Paulo **3**:62, 1908.

32. Splendore, A.: Brasil-med. **23**:361, 1909.

33. Greco, N. V.: Rev. Dermat. **1**:78, 1908.

34. Harter, A., and Gruyer: Compt. rend. Soc. de biol. **61**:309, 1909.

35. Bordes, C.; Berhouet, A., and Errecart, L. M.: Bol. Soc. med. quir. del Centro de la Republica **7**:17, 1934.

36. Talice, R. V.: Ann. de parasitol. **13**:576, 1935.

37. Talice, R. V., and MacKinnon, J. E., in Proceedings of the Third International Congress for Microbiology (1939), 1940, pp. 510-511.

microabscesses in some regions. In addition, the infiltrate in the cutis may consist of plasma cells, young connective tissue cells, many epithelioid cells, lymphocytes, some mast cells and giant cells of the Langhans type. The lymph spaces in the upper third of the cutis are usually dilated.

The granulomatous nature of the lesions is emphasized by the nodular formation. These nodules may be superficial or deep in the cutis. The center of the nodule usually shows necrotic masses or small abscesses, the chronic suppurative zone, and this area is surrounded by richly stained cells consisting of polymorphonuclear neutrophils, eosinophils, lymphocytes, red blood cells and macrophages. Closely adjacent to this area is the tuberculoid zone, which consists of many epithelioid cells and giant cells, varying in number, size and shape and often arranged in tubercle-like fashion. The peripheral, or outer, area of the nodule, the syphiloid zone, is made up of a rich cellular infiltrate of young connective tissue cells, lymphocytes, plasma and mast cells and an increased number of blood vessels, simulating a syphiloid appearance.

The nodules may be distinct and isolated, or several may merge to produce a large granulomatous mass. As a consequence of the granulomatous process, the elastic fibers may become irregular and broken. There may be a perivascular reaction, and the collagen bundles may be invaded by lymphocytes and plasma cells.

Fungus in Tissue.—In tissue or pus the cells of Sporotrichum may been seen as short, blunt, rodlike or spindle-shaped forms, somewhat rectangular, basophilic, measuring 1 to 3 by 2 to 5 microns and occurring singly or in groups. Ovoid to spherical cells may also be seen, and all of these cells may be found, but with difficulty, either freely dispersed in the necrotic material or phagocytosed by mononuclear leukocytes or macrophages. These cells are gram positive and have a colorless, capsule-like periphery.

A second type, designated as the asteroid form, is characterized in tissue or pus by the presence of a radiate structure on the pathogenic fungus. The central cell may be spherical and thick walled (double contoured); it stains pinkish blue with methylene blue and eosin and measures approximately 5 microns in diameter (fig. 3, *4* and *5*). The rays vary in length from 2 to 8 microns and appear similar to those seen in actinomycosis. Acidophilic bodies may be seen throughout the nodule, which are made up of short rays. Other cells may have a mass of pink-staining material, which appears to be emanating directly from the cell wall. The radiating structures are observed within microabscesses surrounded by polymorphonuclear leukocytes and other cells of the inflammatory type.

MADUROMYCOSIS

Definition.—Maduromycosis (mycetoma, Madura foot) is a chronic granulomatous, infectious process localized usually to the extremities but occasionally affecting other parts of the body. It is characterized by variously sized enlargements on the cutaneous surface, which eventually give rise to intercommunicating sinuses and fistulas, from which variously colored granules comprising fungous elements of the genera Actinomyces (Nocardia), Madurella, Monosporium (Allescheria), Indiella, Trichosporium, Aleurisma, Torula, Phialophora, Glenospora, Aspergillus, Penicillium and Sterigmatocystis can be obtained.

History.—Maduromycosis probably dates back many years to the Sanskrit and the missionary writings referred to by Castellani and Chalmers.[38] Most of the present day knowledge of the disease seems to have had its beginning in 1842, when Gill, from the city of Madura, in the Madras Presidency of India, described a condition of the foot which had deformed the extremity with fungoid excrescences, gave off a discharge and affected the joints, ligaments and cartilages. In 1846 Colebrook designated the disease "Madura foot." In 1845 Godfrey, a garrison surgeon in India, found some black granules in the tissues of an amputated foot with the infectious "ulcus grave," a disease which showed ulcers and sinuses with marked swelling. He had referred to this condition in a previous report as "morbus tuberculosis cutis." In 1848 Rustonji differentiated two clinical forms, one with a black substance and the other with yellow granules.

Carter,[39] after careful study, established the mycotic nature of the granules, recognized the black, yellow or white, and red types of granules and created the term "mycetoma." In 1886 he drew attention to the similarity between the granules of actinomycosis and those of mycetoma. Kanthack[40] in 1892 concluded that the yellow and black granules were of the same organism, Actinomyces, but Boyce and Surveyor[41] established these as belonging to two distinct organisms. Their work resulted in the naming of the two disease entities, actinomycotic Madura foot and maduromycosis, as caused by other fungi. There have been a number of reports of cases since the work of these men, and Gammel[42] in 1927 contributed a comprehensive and valuable review of the disease from the standpoint of etiology.

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38. Castellani, A., and Chalmers, A. J.: Manual of Tropical Medicine, ed. 3, New York, William Wood & Company, 1920, p. 2110.

39. Carter, H. V.: Tr. M. & Phys. Soc. Bombay **6**:104, 1861; **7**:206, 1862.

40. Kanthack, A. A.: J. Path. & Bact. **1**:149, 1892.

41. Boyce, R., and Surveyor, N. F.: Phil. Tr., London **185**:1, 1894.

42. Gammel, J. A.: Arch. Dermat. & Syph. **15**:241, 1927.

Pathology.—Grossly, maduromycosis has the appearance of a large tumefaction of varying size and shape. The cutaneous surface shows scarring with ulcerating nodules and fistulas over the enlarged swelling.

The microscopic picture of mycetoma is distinctly granulomatous, with the general picture of active proliferation and infiltration of the various types of cells encountered in other mycotic granulomas. There are local necrosis and abscess formation, followed by the production of fibrous tissue and ultimately scar formation. Some of the abscesses suppurate, while others tend to sclerose. The abscesses may be small and round, or they may coalesce and become large and irregular or elongate. The granules are found within these abscesses, which eventually develop into sinuses and often reach the surface of the skin. The subsequent histopathologic character of the lesions is dependent on the genus and the species of fungus involved. The actinomycotic and aspergillotic lesions have been described. Lesions due to Penicillium, Glenospora and Sterigmatocystis are essentially similar to those produced by Aspergillus. Cases of mycetoma caused by Madurella and to a certain degree by Torula (Phialophora jeanselmei as described by Symmers and Sporer [43]) are essentially similar. In these cases the suppurating abscess is made up of small and large lymphocytes, large mononuclear and polymorphonuclear leukocytes, red blood cells, cellular detritus and albuminoid bodies, all in and around the granules. Some of the small abscesses are made up predominately of lymphocytes and plasma cells, while others contain multinucleated giant cells, surrounded by a thick, sclerotic wall. Plasma cells may be seen in abundance scattered throughout the lesion. The wall of the abscess is made up first with a layer of connective tissue fibrils or large mononucleated cells, which are more or less spheroidal and vacuolated (foam cells), owing to the presence of fats. Surrounding this are a second layer comprising granulation tissue, well vascularized, and then an outer layer of dense connective tissue. Eosinophils have been observed occasionally. Lesions caused by Monosporium (Allescheria) apiospermum seem to lack the fatty histiocytes.

Fungus in Tissue.—In the lesions the fungi causing maduromycosis or mycetoma are seen in the form of grains or granules varying in size, shape and color, depending on the organism responsible for the disease. The granules in the past have been classified according to color. This method is not accurate botanically, since different-colored granules may, on occasion, give rise to similar

43. Symmers, D., and Sporer, A.: Arch. Path. **37**:309, 1944.

organisms. However, from a pathologist's standpoint such a classification may have its merits. Actinomycotic granules may be black, red or yellowish green. Other fungi producing maduromycosis may be listed according to the color of their granules as follows: (1) black granules—Madurella, Glenospora, Torula or Phialophora, Aspergillus and Penicillium; (2) white or yellowish white granules—Monosporium (Allescheria), Indiella, Sterigmatocystis, Cephalosporium and Acremoniella; (3) greenish yellow granules—Aspergillus; (4) red granules—Aspergillus and Rubromadurella.

The nature and the types of actinomycotic granules have been described under the heading "Actinomycosis." The grains of maduromycosis vary according to the fungus and the age of the granule itself. The young granules are in general composed of intertwining septate hyphae and occasionally spherical to ovoid cells, the chlamydospores. Mature granules are made up of a variable number of distinct zones. The innermost zone is made up of mycelial elements, hyphae, chlamydospores, pigmentary granules and disintegrated leukocytic granules. This is surrounded by a deeply pigmented, irregularly amorphous zone. Closely adjacent to this area may be seen radiating hyphae and chlamydospores. Dispersed through the granule are leukocytes in various stages of degeneration. In addition to these usually characteristic zones there may be seen an outer fringe or zone on some granules which is acidophilic and has fine filamentous prolongations, more or less radiate and refractile, or it may consist of a narrow, pink-stained rim, or it may be composed of clubs as seen with Actinomyces. This is the zone of radiation or radiate formation.

Radiate formation on Actinomyces producing mycetoma is fairly common. This same phenomenon is likewise observed not infrequently on other organisms producing maduromycosis. No attempt has been made to cover completely the literature dealing with this disease in order to find the number of cases in which acidophilic material was found on the outer region of the involved granules. The descriptions of granules and the observations reported in some of the papers read, however, seem to indicate that its occurrence is not rare.

Radiate formation on Aspergillus in maduromycosis has been observed by da Fonseca. The granules were greenish yellow. Various species of Madurella when seen in the tissue of mycetoma have likewise revealed radiate formation. De Almeida [44] in 1932 illustrated various granules seen in maduromycosis, several of which were probably Madurella. In one (his fig. 1) he described a thickened and irregular peripheral coating which stained pink with eosin. This no

44. de Almeida, F.: Rev. biol. e hyg. **3-4**:93, 1932.

doubt was radiate formation. In 1926 Gammel, Miskdjian and Thatcher [45] described mycetoma occurring in a 26 year old Mexican born in Texas, which was due to Madurella americana. "The outer zone was a small rim which had a pink tinge." This, too, was radiate formation. In 1938 Hanan and Zurett [46] published observations on mycetoma developing in a white man as a result of wood splinters entering his foot. The black granules in the tissue were formed by the fungus Madurella lackawanna. These authors described four zones in all granules and a fifth zone in some, which was "composed of clubs such as are frequently observed on the periphery of actinomycotic granules."

In 1935 Talice [47] described the red granules of a case of maduromycosis caused by Rubromadurella langeroni. There was a definite zone of radiation, which was both well described and figured as the acidophilic substance of radiate formation. In 1941 Niño [48] published a case of maduromycosis caused by Monosporium apiospermum, in which the yellowish white granules had peripheral structures simulating clubs. Lesions of maduromycosis due to M. apiospermum have been observed in this country with an acidophilic substance on the peripheral structures of the granules which could be considered as radiate formation. Of particular interest is the recent description published by Symmers and Sporer of a mycetoma of the hand which showed black granules. The organism was later identified by Emmons as Phialophora (Torula) jeanselmei. The authors described the granules, in part, as follows, "In some instances clumps of degenerate chlamydospores merge imperceptibly into broad bases composed of acidophilic material, projecting from the border of which are pinkish-staining needle-like formations representing, probably, mycelial elements." A section of the tissue was examined and studied through the courtesy of Dr. Symmers, and it was apparent that the acidophilic material was identical with radiate formation (fig. 4, *1*). In a later publication dealing with maduromycosis as experimentally reproduced in rabbits with granules from the human case of mycetoma, Symmers [49] was able to demonstrate, fifty-eight days after inoculation, granulomatous lesions with the formation of granules. The younger granules showed the same acidophilic material in the form of projections from the granular mass.

45. Gammel, J. A.; Miskdjian, R., and Thatcher, H. S.: Arch. Dermat. & Syph. **13**:66, 1926.

46. Hanan, E. B., and Zurett, S.: Arch. Dermat. & Syph. **37**: 947, 1938.

47. Talice, R. V.: Ann. de parasitol. **13**:584, 1935.

48. Niño, F. L.: Bol. d. Inst. clín. quir., 1941, p. 483.

49. Symmers, D.: Arch. Path. **39**:358, 1945.

It is obvious that most of the genera producing mycetoma are capable of producing granules which give evidence of radiate formation or show acidophilic material at the periphery.

RARE RADIATE FORMATION

There are three other mycoses in which radiate formation has been seen on the causative fungi and which may be considered along with those already described: paracoccidioidal granuloma, chromomycosis and North American blastomycosis. The diseases in which these radiations are found are not uncommon, and it is felt that with careful study more instances of the acidophilic material may very well be described.

PARACOCCIDIOIDAL GRANULOMA

Definition.—Paracoccidioidal granuloma, or South American blastomycosis, or Lutz–Splendore–de Almeida disease, is an acute or chronic granulomatous infection, localized or generalized, involving skin, mucous membranes, lymphatics, internal viscera and bony structures.

History.—The disease was first described by Lutz [50] in 1908, when he considered it a pseudococcidioidal granuloma with the organisms occurring chiefly in giant cells. The fungus was isolated in pure culture from the lymph nodes and salivary glands of the patient. In the same year Carini [51] described a case with primary lesion of the buccal mucosa. In 1909 Splendore described a case of generalized blastomycosis, thus calling attention to two clinical types, the localized, buccal mucosa type and the generalized. He expressed the belief that the two types are caused by two different fungi. Several reports were written by Splendore,[52] and in 1912 he established the specific name of brasiliense for the organism and placed it in the genus Zymonema of de Beurmann and Gougerot.[53] The following year [54] a more lengthy description of the disease was published. Since that time the disease and the organisms have been well described by several investigators, including de Almeida, da Fonseca, Niño, Mazza, Moore and others.

Reference to radiate formation on cells of Paracoccidioides was perhaps first made by Weidman in 1932, when he cited a paper pub-

50. Lutz, A.: Brasil-med. **22**:121 and 141, 1908.

51. Carini, A.: Rev. Soc. de sc., São Paulo **3**:120, 1908.

52. Splendore, A.: Bull. Soc. path. exot. **5**:313, 1912.

53. de Beurmann, L., and Gougerot, H.: Bull. et mém. Soc. méd. d. hôp. de Paris **28**:222, 1909.

54. Splendore, A., in Onore del Prof. Angelo Celli nel 25 anno di insegnamento, Turin, 1913, p. 421.

lished by de Almeida in 1929. Obviously this was a mistake, since what was considered to be denticulate or radiate extensions on this organism have been proved to be multiple gemmation or budding. After Weidman, de Almeida,[55] in his publication of 1934, studied 2 cases of paracoccidioidal granuloma, one of the lung and the other of the skin. In the sections of tissue from these cases de Almeida described what he believed to be true radiate formation.

Pathology.—From the point of view of pathology, paracoccidioidal granuloma, or South American blastomycosis, in many respects mimics North American blastomycosis, or Gilchrist's disease. There is a multiplicity of clinical types and forms with corresponding changes in the histologic response.[56] Grossly there can be seen ulcers, vegetative formations, pustules, papules and tuberculoid and syphiloid manifestations. In systemic disease the lesions are chiefly ulcers, originating mostly in the lymphoid tissue as a result of the lymphatic spread of the organism. The hepatic, the splenic and the uncommon pulmonary lesions are seen generally as nodules and presumably are due to the hematogenous spread of the fungi. Microscopically there is a granulomatous response, such as can be seen in other micotic granulomas, with an infiltrate of lymphocytes, plasma cells, polymorphonuclear leukocytes (many forming abscesses) and giant cells (many phagocytosing organisms), the last often arranged in tubercle-like fashion.

Fungus in Tissue.—In tissue or pus Paracoccidioides is seen as a spherical or ovoid cell varying from 1 to 30 microns in diameter, with a thick wall. The larger cells show simple or multiple budding in the form of minute spherical, ovoid or bacillary gemmules. These buds are arranged on the outer wall of the mother cell and when seen in cut sections appear peripherally and radially arranged to simulate radiate formation. On the other hand, de Almeida observed radiations on the cell wall which gave the appearance of a thickened, irregularly denticulated wall.

An examination of a number of slides of both the cutaneous and the visceral lesions of paracoccidioidal granuloma was finally rewarded by the finding of what could be interpreted as radiate formation. A peculiar phenomenon of Paracoccidioides in tissue seems to be its ability frequently to produce an area of lysis about itself. This area apparently is one of liquefaction and probably results from the lytic action of an enzyme liberated by the fungus in its growth. In paraffin sections the area appears as a clear space. In some of these spaces on the wall of the fungus in hematoxylin and eosin–stained sections one

55. de Almeida, F. P.: Ann. Fac. de med. da Univ. de S. Paulo **10**:163, 1934.
56. Moore, M.: J. Invest. Dermat. **6**:149, 1945.

can see pink-staining extensions in the form of fine prickles or in that of irregular short or elongated formations. These structures, which were considered to be radiations, were seen both on a simple cell and on the multiple budding cells (fig. 4, *2* and *3*).

In examining tissue for radiating structures of Paracoccidioides, there is a phenomenon which may confuse the observer. The fungus is often phagocytosed by giant cells and by large macrophages. The latter give rise to what has been termed pseudococcidioides, which has been observed in pus rather frequently,[57] especially when the fungus involved was Paracoccidioides cerebriformis. When one of these macrophages becomes disintegrated and the fungus set free, bits of the macrophage may adhere to the fungous cells. These particles of clinging macrophage stain with eosin and may resemble radiate formation.

CHROMOMYCOSIS

Definition.—Chromomycosis (chromoblastomycosis, dermatitis verrucosa) is a chronic granulomatous disease affecting the skin chiefly, with rare involvement of regional lymph nodes. The lesions are commonly found on the extremities and may be papular, nodular, verrucous or papillomatous, with or without ulceration and abscess formation.

History.—The discovery of chromomycosis was made by Pedroso in 1911. Reporting from Brazil, he described a "black blastomycosis" due to dark brown cells which were seen in sections of the diseased tissue. In 1920 Pedroso and Gomes[58] published this case with 3 others from Brazil and named the fungus Phialophora verrucosa on the basis of a case reported in 1915 by Lane[59] and also by Medlar,[60] the organism in that case having been described as P. verrucosa by Thaxter. Since 1920 numerous cases have been reported from many parts of the world. These have been reviewed by de Almeida,[61] Moore and Mapother[62] and Weidman and Rosenthal.[63] In 1942 Pardo-Castello, Leon and Trespalacios[64] reported 31 cases from Cuba and included a fine description of the clinical types of chromomycosis.

57. Moore, M.: Arch. Dermat. & Syph. **38**:163, 1938.

58. Pedroso, A., and Gomes, J. M.: Ann. paulist. de med. e cir. **11**:53, 1930.

59. Lane, C. G.: J. Cutan. Dis. **33**:840, 1915.

60. Medlar, E. M.: Mycologia **7**:200, 1915.

61. de Almeida, F. P.: Mycologia medica: Estudo das mycoses humanas e de seus cogumelos, São Paulo, Companhia Melhoramentos, 1930, p. 583.

62. Moore, M., and Mapother, P.: Arch. Dermat. & Syph. **41**:42, 1940.

63. Weidman, F. D., and Rosenthal, L. H.: Arch. Dermat. & Syph. **43**:62, 1941.

64. Pardo-Castello, V.; Rio Leon, E., and Trespalacios, F.: Arch. Dermat. & Syph. **45**:19, 1942.

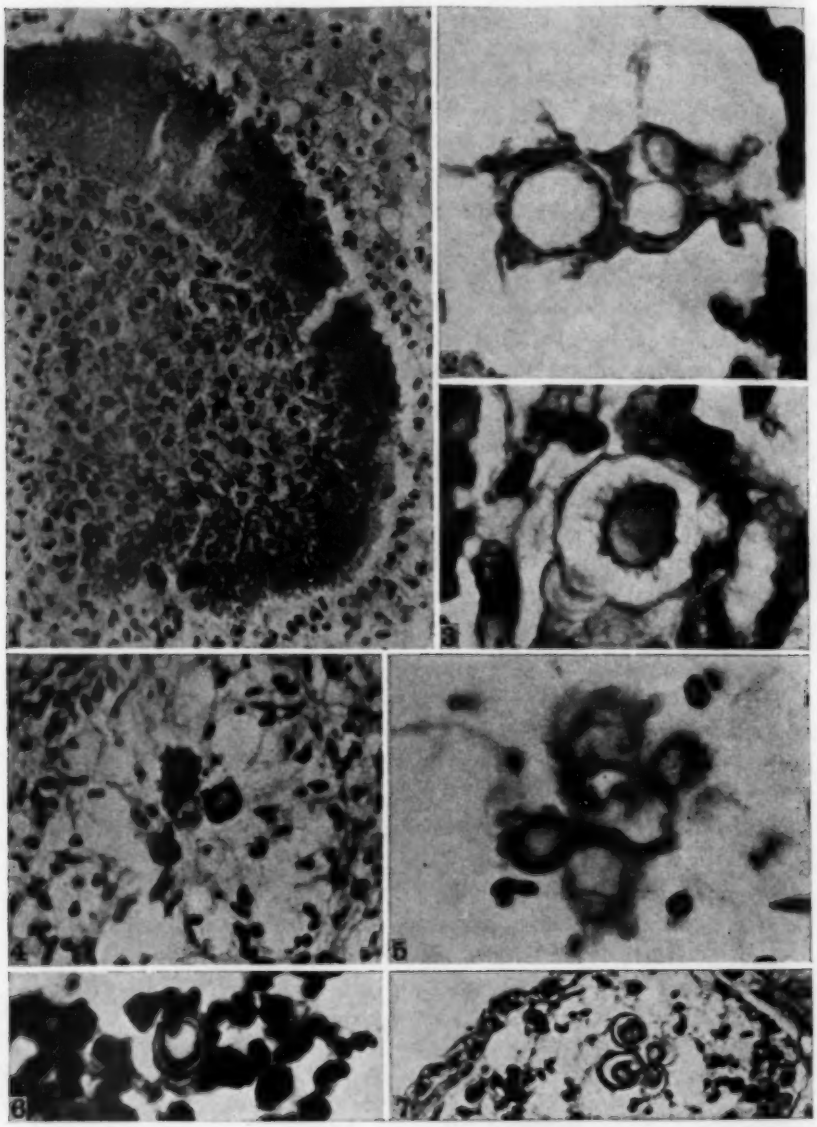

Fig. 4.—*1*, Section of granule of Phialophora (Torula) jeanselmei showing fringe of acidophilic material; case of Drs. Symmers and Sporer; hematoxylin and eosin; × 361. *2*. Paracoccidioides brasiliensis with multiple budding and acidophilic material; hematoxylin and eosin; × 1,110. *3*. P. brasiliensis showing acidophilic radiate-like substance in tissue; hematoxylin and eosin; × 1,110. *4*. Fungous cells of chromomycosis with acidophilic incrustations in tissue; hematoxylin and eosin; × 425. *5*. Fungous cells of chromomycosis with irregular peripheral radiations in tissue; hematoxylin and eosin; × 1,110. *6*. Fungous cell of blastomycosis with acidophilic substance partially involving the wall, seen in a pulmonary nodule; hematoxylin and eosin; × 648. *7*. Fungi of blastomycosis showing adherent acidophilic material on cell walls, seen in a cutaneous abscess; hematoxylin and eosin; × 425.

In 1943 Moore, Cooper and Weiss [65] reviewed the cases reported in the United States.

The first report of radiate formation on cells of fungi producing chromomycosis was made by de Almeida in 1934. De Almeida observed the radiate structures in tissue furnished him by Maciel from a case which occurred in Santos, Brazil, in 1916. The second case was that of a patient in a hospital in São Paulo, Brazil. In addition to these 2 cases, the diagnosis of which I have confirmed, there has been a third case reported from Brazil in which the characteristic radiations were present on the cells.

Pathology.—Grossly, chromomycosis manifests itself by the production of various types of lesions of the skin, chiefly of the extremities but also of the face, the ear, the neck, the chest, the shoulders and the buttocks. On the basis of the cases reported in the literature and 31 cases observed in Cuba, Pardo-Castello and his associates have classified chromomycosis into five types: the verrucous or papillomatous, the tuberculoid, the syphiloid, the psoriasiform and the elephantiasic. Briefly these may be described as follows: 1. The verrucous or papillomatous type exhibits first papules and then nodules, which enlarge to show eventually abscesses, rarely suppuration, and finally central healing and scar formation. 2. The tuberculoid type shows small patches or nodules with erythematous areolas. 3. The syphiloid form in the early stages is nodular and scaly, with some erythema, and then becomes ulcerated and covered with crusts. The nodules are small, in the early lesions, and are flattened, serpiginous, annular or arcuate in their arrangement. 4. The psoriasiform type is characterized by superficial inflammatory lesions with infiltration, covered with thick, adherent white scales. 5. In the elephantiasic form the extremities are greatly enlarged, and there are many features characteristic of the other types with the addition of cicatrization.

Microscopically, the lesions of chromomycosis have a number of features in common with other mycotic granulomas. The epidermis is hyperplastic, showing hyperkeratosis and acanthosis. Within the elongated rete pegs may be seen microscopic abscesses filled with polymorphonuclear leukocytes, cellular debris and fungi. Within these areas may also be seen Langhans giant cells phagocytosing fungi.

The dermis responds most strongly to the organism. There are edema, pronounced cellular infiltration and, in older lesions, evidence of fibrosis. The infiltrate consists of polymorphonuclear leukocytes, lymphocytes, plasma and epithelioid cells, eosinophils, Russell's fuchsin bodies, macrophages and giant cells of the foreign body or Langhans

65. Moore, M.; Cooper, Z. K., and Weiss, R. S.: J. A. M. A. **122**:1237, 1943.

type, the last sometimes arranged in tubercle-like fashion. In the early lesions there is an extensive infiltration in which cells of the types just mentioned participate, accompanied by thickening, edema, hyperplasia, hyperkeratosis and acanthosis. Granulomatous changes are not apparent, and fibrosis, which is most noticeable in older lesions, is lacking. Scattered throughout the rich cellular infiltrate, chiefly polymorphonuclear leukocytes, can be seen the thick-walled cells of the fungus. The older lesions show some necrosis and abscess formation, but these are not as pronounced as in Gilchrist's disease. The fungus can be seen also in the giant cells and in the abscesses.

Fungus in Tissue.—In tissue or pus the large sclerotic cells of the fungus are dark brown, thick walled and spherical or irregular in outline; they may be single, multiple or multiocular and are approximately 3 to 10 microns in diameter. They reproduce by enlargement and cross wall development to form mulberry-like clusters, but never by budding. In old, necrotic lesions there may be seen short filaments, which are germinations of the spherical, sclerotic cells.

Radiate formation on the dark brown, thick-walled cells, as on the cells of Paracoccidioides, is seen as an eosinophilic or acidophilic corona on the wall of the fungus (fig. 4, *4* and *5*). The acidophilic material, however, is not uniform in appearance, being thicker on some parts of the cells and lacking on other parts. The projections may extend outward either as an irregular mass or as somewhat rounded elongations, varying in length. From their appearance, staining quality and location, they should be considered as radiate formation.

NORTH AMERICAN BLASTOMYCOSIS, OR GILCHRIST'S DISEASE

Definition.—Blastomycosis is a granulomatous, infectious process which is protean in its manifestations and caused by budding, thick-walled, yeastlike organisms.

History.—The first case of blastomycosis was reported by Gilchrist in 1894 at the session of the American Dermatological Association. He found yeastlike bodies in lesions described by the attending physician as scrofuloderma. Two years later [66] this case was published in detail. In 1896 Curtis [67] reported a similar organism from a myxomatous tumor of the leg. In the same year, Gilchrist and Stokes [68] published a short paper on a second case of blastomycosis, and this was published

66. Gilchrist, T. C.: Johns Hopkins Hosp. Rep. **1**:269, 1896.

67. Curtis, F.: Ann. Inst. Pasteur **10**:449, 1896.

68. Gilchrist, T. C., and Stokes, W. R.: Bull. Johns Hopkins Hosp. **7**:129, 1896.

in detail in 1898.[69] The observations of Hyde and Montgomery on the clinical, pathologic and mycologic aspects of the cutaneous lesions of the disease were published by Montgomery[70] in 1902 in an article which is a masterpiece.

Pathology.—Blastomycosis closely resembles tuberculosis, a neoplasm or syphilis. In the skin are seen pustules, ulcerations, nodules, gummas and papillomas, granulomatous in nature. In the internal organs the disease manifests itself as miliary or large-sized nodules, abscesses and neoplasm-like formations.

Microscopically, the epithelium is irregular, thickened and elevated in parts and thin and depressed in others. The stratum corneum may be lacking in some places and hyperkeratotic in others. The epidermis is hyperplastic, with long extensions into the corium. Many of the pseudoepitheliomatous proliferations contain the miliary abscesses characteristic of the disease. The abscesses are widespread throughout the epithelium, vary in size and number and are made up of epithelial detritus, leukocytes (some in various stages of degeneration), epithelial cells, nuclear fragments, red blood cells, giant cells of the Langhans type and budding, yeastlike cells of the fungus. The abscesses are surrounded by flattened, apparently functionless epithelial cells, which form a type of nest. The rete is usually edematous and infiltrated by leukocytes. Cornified cells may be seen as isolated forms, in groups or in whorls, and the giant cells, occasionally surrounded by a few leukocytes, may be seen in the dermis, occurring singly or arranged in tuberculoid fashion.

In the corium one may find the type of abscesses seen in the epithelium. There are inflammatory changes, which may be subacute, acute or chronic. The infiltrate, which may be perivascular, with the vessels hyperplastic, is made up of leukocytes, plasma cells and young connective tissue cells, varying in density. Mast cells and giant cells may also be seen. Plasma cells, giant cells and new connective tissue cells may occasionally show hyaline degeneration, and densely infiltrated areas show complete destruction of the collagen. Tubercle-like formation may also be seen.

Fungus in Tissue.—In tissue the fungus is seen usually in the miliary abscesses, in the epithelium and in the corium, always surrounded by an inflammatory process. The number of cells of the fungus is not constant and varies in these locations. Occasionally, an area with many giant cells and abscesses may show one or two fungous cells, whereas other areas with few abscesses or giant cells

69. Gilchrist, T. C., and Stokes, W. R.: J. Exper. Med. **3**:53, 1898.

70. Montgomery, F. H.: J. A. M. A. **38**:1486, 1902.

may show many budding, yeastlike organisms. The fungus is seen as single or budding, yeastlike cells, thick walled, having a double-contoured appearance and measuring approximately 5 to 12 microns in diameter. In old, necrotic lesions one may see simple branching cells, which may attain a length of 20 microns.

As in the case of chromomycosis and that of South American blastomycosis, eosinophilic or acidophilic material may be rarely seen on the walls of the fungous cells. In the few observations of this type of formation, the pink-staining substance was noted only on a portion of the cell wall as an irregular projection which extended a short distance from the wall (fig. 4, *6* and *7*). The substance did not have the size of that noted on fungi of chromomycosis and paracoccidioidal granuloma.

OTHER RADIATE FORMS

In addition to the diseases mentioned, there are others in which radiation on the infecting organism has been described. On rare occasions the radiate forms have been noted, chiefly in animals but also in man, on such organisms as Mycobacterium tuberculosis and on Staphylococcus as seen in the disease botryomycosis. In 1919 Magrou,[71] while discussing the actinomycetoid form of botryomycotic granules, presented evidence of radiation occurring on Monilia albicans in an experimentally infected rabbit. The organism had been obtained from human sputum. Radiate forms were observed in the kidney, where numerous inflammatory foci were present. Radiate forms of M. (Candida) albicans in man have not been reported in the literature. The organism of actinobacillosis in animals has also shown the radiation effect. Ravaut and Pinoy[72] have demonstrated this phenomenon on Actinobacillus in man.

The presentation of radiation effects on fungi in tissue would perhaps be incomplete without a discussion of other phenomena, chiefly gloea and capsule formation, but also granular projections, occurring on fungous cells in tissue.

Gloea Formation.—The production of gloea is perhaps more common with bacteria. Fungi in the pathogenic state affecting man can form gloea in a limited number of instances. This is evident in the hair diseases known as piedra (piedra nostras, trichosporosis), both the Brazilian and the Colombian type (fig. 5, *1*), and lepothrix (trichomycosis axillaris) (fig. 5, *2*). The fungi grow and surround the hair in the form of nodules or masses, the elements of which seem to be held together by a mucilaginous or gelatinous matrix, the gloea. In cultures

71. Magrou, J.: Ann. de l'inst. Pasteur **33**:344, 1919.

72. Ravaut, P., and Pinoy, E.: Ann. de dermat. et syph. **10**:417, 1909.

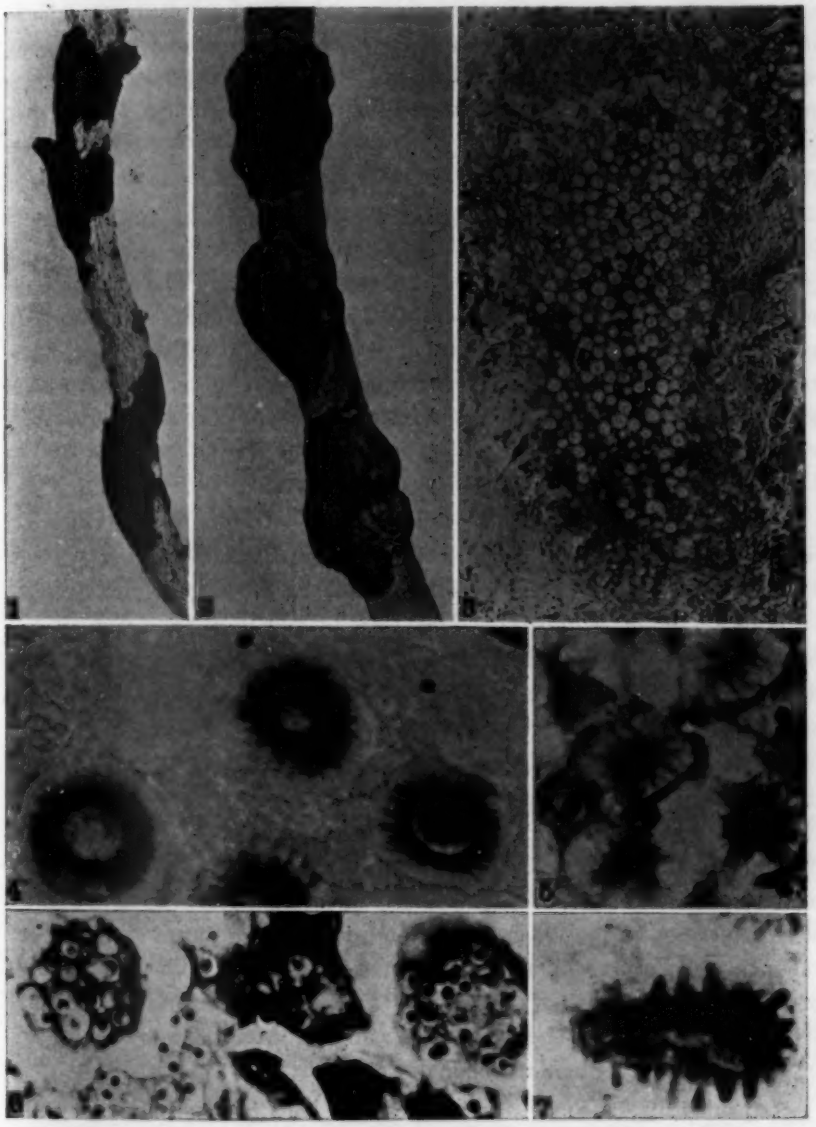

Fig. 5.—*1.* Hair with nodules of piedra; × 53. The gloea has hardened to form pebble-like concretions. *2.* Nodules of lepothrix on a hair; × 74. The gloea remains mucoid, and the granule consequently is soft. *3.* Cyst-like lesion of the brain showing mucoid encapsulated cells of Cryptococcus forming a jelly-like mass; hematoxylin and eosin; × 79.5. *4.* Cells of Cryptococcus showing radiating extensions of the cell walls; hematoxylin and eosin; × 956. *5.* Irregular cell wall extensions of Cryptococcus; hematoxylin and eosin; × 398.5. *6.* Histoplasma capsulatum in reticuloendothelial histiocytes with clear mucoid capsules; hematoxylin and eosin; × 919. *7.* Piriform cell of Histoplasma grown on agar with the characteristic cell wall extensions; iron alum-hematoxylin stain; × 1,222.

on artificial mediums, certain fungi are capable of producing a gloea-like substance, which is liberated with spores from an endogenous spore-forming organ and serves to hold the spores together until they reach full maturity. The gloea disintegrates, and the spores are set free. Among pathogenic fungi a notable example of this phenomenon can be found with P. verrucosa, an etiologic agent of chromomycosis.

Capsule Formation.—In contrast to gloea, which holds the fungus elements together *en masse,* the capsule is a mucoid, mucilaginous or gelatinous substance surrounding the individual cell. The capsules do not stain with the ordinary dyes. When seen in fungous groups or masses, the capsular material may appear as gloea, and the whole may resemble a jelly-like, gelatinous or mucoid cyst (fig. 5, *3*). An outstanding example of this is seen in lesions of cryptococcosis (torulosis), especially in those involving the brain. The causative organism is Cryptococcus hominis (Cryptococcus neoformans).

Of particular interest with regard to C. hominis is the fact that on occasion one can see encapsulated forms with radiations extending from the cell wall into the capsule, usually to the edge of the mucoid substance (fig. 5, *4* and *5*). This type of radiation, however, is not identical with that described for such organisms as Actinomyces, Coccidioides or Sporotrichum, since the rays are definitely continuous with, or are a part of, the cell and stain identically.

Another well known example of capsule formation is that seen on cells of Histoplasma capsulatum in tissue. The capsules vary in thickness and, like those of C. hominis, do not stain ordinarily (fig. 5, *6*). In 1940 Henrici [73] suggested the possibility of radiation or actinomycetoid formation with H. capsulatum. It is probable that he was referring to the tuberculate condition of the large spherical to piriform cells of Histoplasma in culture (fig. 5, *7*). These structures are functionless tubelike projections which are part of, and extend from, the cell wall. They resemble radiate formation and should not be confused with the acidophilic material.

Capsules may also be seen, under varying conditions and in various organs, on such yeastlike organisms as M. (Candida) albicans, Zymonema (Blastomyces) dermatitidis, Paracoccidioides brasiliensis and P. cerebriformis and others. In fact, capsule formation is not limited to tissue but may be induced under certain conditions of cultural growth. Huge capsules were formed on cells of P. cerebriformis when the organism was grown on wort agar.[57]

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73. Henrici, A. T.: J. Bact. **39**:113, 1940.

Cell Wall Extensions.—Many fungi when grown on artificial mediums produce various types of spores, such as ascospores, conidia, macroconidia or fuseaux, chlamydospores and others, which may have as a characteristic echinulate, granular or radiating projections developing from the spore wall. Spores of species of the genera Aspergillus, Scopulariopsis, Ustilago and others show small spines and are said to be echinulate. Although characteristically seen in cultures, as in the case of the first two genera, and in the parasitic phase on plants, as in the case of Ustilago, such spiny spores may be seen in parasitized human tissue. This is simply because the fungi in this case may be considered as secondary invaders and perhaps live as saprophytes in the tissue. Although producing disease in man, the fungi are not capable of adapting themselves to a parasitic form, such as is found with many of the pathogenic fungi. The fuseaux or macrospores of Microsporum, Trichophyton and Epidermophyton, the so-called dermatophytes, may show granular formations. These spores, however, are limited to cultures. Various organisms of the Dematiaceae, as well as some of the Phycomycetes, may show in culture, on the spores and the filaments, granular projections or incrustations.

COMMENT

Radiate formation on fungi in tissue is not a species-specific or genus-specific characteristic or phenomenon. Such fungi, referred to as actinophytes by Lignières and Spitz,[74] may include, as indicated by Magrou, many genera and species. Except for such organisms as A. bovis or israeli, and perhaps C. immitis, with which radiate formation is commonly associated, this structure in human tissue is sufficiently rare to be given further consideration here. It is worthy of note also that radiations do not always occur on Actinomyces and Coccidioides, for one may study tissue from many cases and find, first, that not all the sections will exhibit radiation forms and, secondly, that in the same sections some organisms will be actinomycetoid while others will lack the acidophilic substance. This inconstancy is of course greatly accentuated in the other diseases listed in this paper in which the radiation effect is not common. The finding of radiations with so many varied organisms would indicate that this formation is a concomitant finding frequently associated with the organisms in tissue.

The type and the form of the acidophilic substance have led to a great deal of speculation as to the nature and the source of this material.

74. Lignières, J., and Spitz, G.: Rev. Soc. méd. argent. **10**:5, 1902; Arch. de parasitol. **7**:428, 1903.

A brief review of the types of radiation should be interesting. In the case of actinomycosis, reference is generally made to the club formation. This means that the terminal portion of the radiating structure is broader than that part which is closer to the fungous cell or mass. In some cases, not all, it has been possible to demonstrate filaments of Actinomyces within these rays, and Weidman has described the structure in these cases as follows, "the hyaloid incrustment being continued like a coat of ice over the surface of the colony and its projecting terminal hyphae." In some granules of Actinomyces the acidophilic substance seems to be in the form of irregular projections, lobulated masses and unequally thickened coverings. In the case of aspergillosis the radiations do not usually appear club shaped, but rather take the form of elongated crystals, with short, angular projections and with "broken-off" or abrupt tips. They may also be somewhat cylindric, as described by Wiedman, uniform or irregular in size, appearing at times like a mass of crystalline needles. There is in addition another type which appears to be in the form of an emanating substance on the hyphae and which has been compared to the Hülle cell seen on aborted reproductive structures of Aspergillus. On C. immitis may be seen prickles of uniform size and appearance, extending from the wall of the mother cell, irregular radiating masses, and even extremely long filiform or tubular rays, as described by de Almeida. Club formation may also be seen on occasion. On cells of S. schencki the rays take on an asteroid or a star-shaped arrangement, are somewhat tubular in appearance and may be of equal or unequal length. In the case of maduromycosis the radiation may be of the type seen on Aspergillus or of the actinomycetoid form. As regards paracoccidioidal granuloma, chromomycosis and blastomycosis, the radiation effect is seen as an acidophilic substance of nonuniform thickness either completely or incompletely involving the cell wall of the fungus.

It is apparent that the form of the radiations varies considerably with the organism and with the disease. An explanation for this may perhaps be found in the type of pathologic condition that the fungus produces. All of the diseases listed have in common leukocytic infiltration of a variable degree, inflammation of a varying degree and a terminal granulomatous response, or, as preferred by some, a chronic, progressive inflammatory response. In some diseases, particularly actinomycosis, maduromycosis and to a lesser degree coccidioidal granuloma, there is evidence of marked suppuration with sinuses being formed and pus constantly flowing to either the surface of the body or to adjacent tissues. In aspergillosis suppuration and large abscess or even cavity formation are present, but the flow of pus is not of the

rapidly spreading type seen in actinomycosis. The process may be compared with a stagnant pool which increases in size simply by erosion and disintegration of its surroundings. In sporotrichosis, blastomycosis, paracoccidioidal granuloma and chromomycosis, suppuration may be noted, but here too the flow of the pyogenic debris is slow and again results in abscesses of varying size. Sinuses are rare in these diseases. The variation in the type of rays may, therefore, depend on the motility of the fluid surrounding the organism. It is known that a constantly flowing fluid will tend to round up the tips exposed to the current and that, on the other hand, crystals with sharp edges and needle-like formations will develop in a quiescent fluid. Such an explanation may seen plausible for actinomycotic and aspergillotic granules, but the appearance of the small amount of the incrusting substance or radiate formation on the organisms of chromomycosis, paracoccidioidal granuloma and blastomycosis would be difficult to explain on this basis alone.

The nature and the source of the rays present perhaps the most intriguing problem. Unfortunately, the amount of experimental work done toward solving this phase has been limited by the amount of available material. With the knowledge that these structures can be produced regularly in animals, however, the amount of material can be greatly enlarged, so that an answer should be forthcoming before long. Be that as it may, the theories advanced as to the nature and the source of the radiations have been many and confusing.

Lichtheim in 1882 suggested that the rays were aborted productions of the fungus. Boyce in 1893 used various stains on the aspergillotic granules and concluded that the rays were distinct cell elements. Rénon in 1897 agreed with Lichtheim that they were aborted fungous productions but also pointed out that the granules represented the index of the extreme defense of the body and the lowered resistance of the fungus. In other words, this radiation phenomenon could be interpreted to mean either a defense mechanism set up by the fungus or an offense set up by the body. Lignières and Spitz, supported by Brumpt, regarded the radiate formation as "young protoplasm, capable of budding and serving as nutrient for the filaments in the interior of the grain." On the other hand, Ravaut and Pinoy considered the actinomycotic masses as the result of a mixed production depending on parasite and host. They compared the formation of masses to that of the hold-fasts of certain fungi parasitic on plants (Peronosporaceae).

Pinoy [75] expressed the belief that the radiate formation is a result of the digestive action of the host on the membrane of the fungus.

75. Pinoy, E.: Bull. Inst. Pasteur **11**:929 and 977, 1913.

Magrou felt that the actinomycotic form resulted from the parasitic life of the organism—a defensive reaction of the leukocytes and of the humors which accumulate around the parasite. In other words, the radiate forms have attained a "state of symbiosis with the leukocytes of the vertebrates."

Bayne-Jones [76] in 1925 presented the two theories advanced as to the nature of clubs. One attributed to the rays a developmental part in the life cycle of organism (theories of Boyce and, especially, of Lignières and Spitz, supported by Brumpt). The other theory regarded the club as a thickening of the sheath enclosing the filament to protect it against the effect of animal fluids (Rénon). Bayne-Jones did not believe that the club seen with Actinomyces was exclusively the result of interaction between the organism and the animal fluids (theories of Ravaut and Pinoy and of Magrou). He held that club formation could be produced in simple mediums free from serum and other animal protein. This was in contrast to the work of Wright,[77] who obtained granules (showing radiate formation) with A. israeli on broth to which had been added organic liquids, including blood, serum and pleural fluid. In 1 per cent dextrose–meat infusion agar and 1 per cent dextrose–meat infusion broth the filaments at the edges of the colonies were enclosed in sheaths of hyaline material, which terminated in bulbous thickenings over the ends of the filaments. The bulbous portion took no part in the growth as seen in hanging drop preparations. Growth of the filament took place away from the bulbous end, which apparently did not change. Similarly, Langeron, Cauchemez and Alleaux [78] were able to produce radiate forms of Actinobacillus on Sabouraud's dextrose agar without organic products, confirming Bayne-Jones' work, and in disagreement with the results of Ravaut and Pinoy, who obtained granules of Actinobacillus by growing the organism on a medium containing peptone and dextrose to which was added beef serum.

A different view regarding radiate formation was initiated by Ahlfeldt when she noticed that the prickles were found only on the adult organism of C. immitis when it was ready to liberate the young forms. This idea was taken up by Weidman, at least as regards C. immitis, who pointed out, in view of Ahlfeldt's observation, that radiation may be an accompaniment of reproductive processes when the reproductive structure is fully developed. It should be pointed out that this is not a rule and that many more mature cells have been

76. Bayne-Jones, S.: J. Bact. **10**:569, 1925.

77. Wright, J. H.: J. M. Research **13**:349, 1905.

78. Langeron, M.; Cauchemez, L., and Alleaux, V.: Ann. de parasitol **3**:225, 1925.

observed without the prickles or radiate formation than have been seen with them. Especially is this true of the fungus seen in the skin. It seems unlikely that the radiation effect is part of the process.

Nicaud [79] in 1928, while discussing the actinomycetoid form of A. fumigatus, revived some of the older theories when he concluded that "some organisms obtain an actinomycetoid form simply because of the associated growth of certain bacteria." Secondly, he pointed out that "in the case of Aspergillus radiations, this is a result of the influence of the medium, the reaction to the cells of the humors which perhaps modify by a digestive action the peripheral elements of the parasite, but it concerns elements which have conserved their vitality." This seems to fit in with the theories of Pinoy and Ravaut and Pinoy.

In 1932 Weidman [19] analyzed much of the work already published and presented a discussion of radiate formation on a probable Aspergillus occurring in an infected capybara. In trying to ascertain whether the radiate formation was a product of the fungus or a contribution from the inflammatory processes involved, Weidman made careful studies of stained preparations from the animal. "In any event, the hyaloid material comprising the ray extended into direct contact with the cell in the interior of the fungus. Indeed, conditions were sometimes so favorable that the substance could be observed becoming integral with the fungicellulose in the wall of the micro-organism itself. I feel very strongly that this material is fungus in production and not host tissue." Haidenhain's iron-hematoxylin stain was also used by Weidman. This staining method colored the hyaloid substance black, and the wall of the cell took the red counterstain. "Again, the hyaloid incrustation was found to come into most intimate contact with the wall of the cell. However, the two did not blend; at least, in some cases the wall of the cell was recognizable as a pink, double-contoured shell independent of the rays." This appears to be a contradiction of the earlier statement. Later in the paper the author inclines to the view, "that the incrustation represents suppressed formations of the order of the Hülle cells (p. 739). . . . it is tempting, indeed, to assume that products of the micro-organism have diffused outward and coagulated or otherwise hyalinized surrounding fluids."

The incrustations of Aspergillus as described under the heading "Aspergillosis" do not appear to be "of the order of Hülle cells," as mentioned by Weidman. In the first place, these structures may occur anywhere on the filaments or hyphae and need not be limited to the terminal cells of the fungus, as is characteristic of Hülle cells. Secondly,

79. Nicaud, P.: Compt. rend. Soc. de biol. 99:1565, 1928.

they may occur as isolated nodules on the hypha, as interrupted nodules, or they may extend for some distance as a continuous sheath. They vary in size and shape. In fact, they are strongly reminiscent of the nodules of piedra on the hair. From their appearance alone they suggest incrustations.

Meyer [80] in 1934 concluded that the radiate formation on Actinomyces is not a form of degeneration or a defense mechanism set up by the organism, but a product of the host-parasite reaction. He favors Weidman's hypothesis that the radiate formation is produced by materials emanating from the organism. The bluish or pale violet tint observed in the granules he interprets as a lipid coming from the mycelium.

One of the most recent theories regarding radiate formation is that promulgated by Henrici [74] in 1940. Henrici was attempting to obtain data on the mechanism of infection in deep-seated mycoses by producing aspergillosis experimentally in rabbits. He found that death could occur in these animals at two definite periods of time. In one period, within two days, death may follow inoculation with large doses of organisms and is attributed to an endotoxin produced by the fungus. In the second period, ten days or longer after inoculation, death results when the lesions change from abscesses to tubercles—a time when the animals have become hypersensitive to products of the fungus. He concluded from his observations "that after the infection has persisted for seven days or more, something happens which leads simultaneously to hypersensitivity; to an altered response of the host tissues, the tubercle; to an altered morphology of the fungus, the actinomycetoid form; and to a dissemination of the disease to new areas." He continues, "Although not yet proved, it seems a justifiable assumption that in experimental aspergillosis the actinomycetoid form of the fungus and the tuberculoid character of the lesion result somehow from the allergic state which develops." This is a new approach to the possible explanation of radiate formation. It is quite likely that the mechanism, whatever it may be, and the nature of the radiate material are essentially the same for all the diseases described in this paper.

In the absence of positive proof it is of course difficult to accept fully any one theory. One of the ideas set forth by the various authors may prove eventually to be the correct one.

Since the nature and the source of the radiating material are still in a theoretic or speculative stage, it is a temptation to add another possibility to the already long list. The work of Menkin [81] on the dynamics of inflammation has brought to light a substance described as

80. Meyer, K.: Compt. rend. Soc. de biol. **115**:1684, 1934.

81. Menkin, V.: Dynamics of Inflammation: An Inquiry into the Mechanism of Infectious Processes, New York, The Macmillan Company, 1940, p. 37.

leukotaxine which can be isolated from inflammatory exudates. This substance has the property of attracting leukocytes (leukotactic), according to Menkin. When leukotaxine and the acidophilic substance are compared, there are many features of both which suggest a relationship. Both are crystalline material (doubly refractile) of a yellowish to brownish color. Both are insoluble in hydrochloric acid. According to Menkin, leukotaxine is soluble in glacial acetic and nitric acids. According to Israel, the radiating substance of the actinomycotic granule is insoluble in sulfuric and acetic acids. Both substances are insoluble in ether, chloroform or absolute alcohol. Heat does not effect either. Hydrolyzing of leukotaxine for about twenty hours in 9.5 normal sodium hydroxide inactivated it, whereas soaking of the granules of actinomycosis in alkali robbed the granules of their gloss and made them paler. Soaking of granules of aspergillosis for several days in 10 per cent potassium hydroxide changed the radiating substance, according to Weidman, to a finely granular matrix.

In view of the similar characteristics described it would seem logical to assume that the acidophilic substance is similar to or a form of leukotaxine. From a purely observational standpoint there are several points worthy of note. Chief among these are the facts that both substances are present in an inflammatory response and that leukocytes are found in abundance where both are concerned. Leukotaxine is a diffusable substance, and the radiate material tends to become a solid incrustation. This may be explained on the basis that the leukotaxine may become hardened as a result of its interaction with the fungous cell. On the other hand, diffusable material may crystallize about and on a foreign body, as is apparent in the initiation of crystal formation in mother liquor. Fungi, as has been pointed out in another publication,[62] may be considered as actively proliferating foreign bodies.

In line with this approach to an understanding of the acidophilic substance, Berger, Vallée and Vézina[82] favored the hypothesis that the radiating material was derived from the inflamed tissue, stating that "the clubs are not a direct result or product of the pathogenic agent, but seem to arise through a peculiar interaction between the agent and the surrounding exudative elements." In support of their belief they cited the work of Levaditi and Dimancesco-Nicolau,[83] who in 1926 obtained radiate formation in an experimental animal by injecting an oily suspension of tellurium intramuscularly. Sections of the muscle, ninety-nine days later, showed granulomas with masses of

82. Berger, L.; Vallée, A., and Vézina, C.: Arch. Path. **21**:273, 1936.

83. Levaditi, C., and Dimancesco-Nicolau, O.: Compt. rend. Soc. de biol. **95**: 531, 1926.

the injected inorganic substance surrounded by club formation. The radiate material appeared similar to that seen on actinomycotic granules and had the same tinctorial qualities. This work, of course, favors the theory that the radiations are produced by the host.

It seems justifiable, therefore, to study further the possible relationship of radiate formation and leukotaxine.

SUMMARY AND CONCLUSIONS

The phenomenon of radiate formation usually associated with actinomycosis, and consequently termed the actinomycetoid form of radiation, has been observed on fungi in human tissue. It is commonly encountered in actinomycosis, frequently observed in coccidioidal granuloma and occasionally noted in aspergillosis; it has been observed also in sporotrichosis, maduromycosis, paracoccidioidal granuloma, chromomycosis and blastomycosis. Pathologic study of the mycoses with which the radiation effect is associated reveals that in all cases there is a granulomatous response of the tissue accompanied by a variable degree of suppuration, which is made manifest in some diseases, notably in actinomycosis and maduromycosis and to a lesser degree in coccidioidal granuloma and aspergillosis, by the formation of sinuses. In all instances there is a marked inflammatory reaction of the tissue, with massing of leukocytes in close association with the radiate form.

The radiating structures vary somewhat in shape and size. They may be of the actinomycetoid type, i.e., in the form of clubs or rays with broadened tips; they may assume the form of prickles or crystalline needles, tubular in appearance; they may appear as somewhat flattened hyaloid extensions, doubly refractile, with "broken-off" tips and lateral angular projections, or they may be seen as peripheral nondescript, short extensions either partly or wholly surrounding the fungus. The unstained material is hyaloid, crystalline or ground-glass-like in appearance and yellow to yellowish green in color. These rays stain pink with eosin and consequently have been referred to as acidophilic substance.

Radiate formation is not a specific characteristic of the genus or of the species but may be found associated, with a varying degree of frequency, with any mycosis which produces an inflammatory reaction in the tissue that tends to persist and consequently becomes chronic and results in a granulomatous response.

The nature and the source of the radiating substance have not been definitely established. Several theories have been advanced in this regard, and these briefly are as follows: 1. The radiating mate-

rial is an aborted product of the fungus. 2. It is living protoplasm capable of multiplying. 3. It is the result of a host-parasite relationship—also a result of the digestive action of the host on the membrane of the fungus. 4. It is a protective mechanism set up by the fungus (a diffusion product). 5. It is an accompaniment of a reproductive process. 6. It is the result of an associated growth of certain bacteria. 7. It results from the allergic state established by the organism in the tissue. To these is added another possibility, namely, that the radiate substance may be similar or related to leukotaxine, described by Menkin.

In addition to radiate formation, other phenomena have been noted on fungi in human tissue. They consist of capsule formation as noted particularly on cells of Cryptococcus and Histoplasma and gloea formation, such as is found in hair infections (piedra and lepothrix). Also, cell wall projections can be seen on various spores both in tissue and in culture.

THE LEUKOPENIC FACTOR OF EXUDATES

The Mechanism Concerned in the Leukopenia Induced by It

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IN A recent communication I demonstrated that exudates contain a leukopenic factor, particularly if at a p_H indicating acidity. On injection of such exudates sharp leukopenia ensues. This is subsequently followed by leukocytosis. The latter is to some extent referable to a leukocytosis-promoting factor (abbreviated as LPF) present in exudative material.[1] The leukopenia occurs rapidly and lasts only several hours.[2] The fact that such a factor is liberated at the site of an acute inflammation may be of significance in explaining numerous leukopenic states accompanying some well known inflammatory processes.

The question arises as to the mechanism of the leukopenia which develops after the injection of the leukopenic factor of exudates. The factor is often, though not exclusively, found to be in close association with pyrexin, the pyrogenic factor which is present in exudates and which per se offers a reasonable explanation of the mechanism of the fever produced with inflammation.[3] The present communication endeavors to throw further light on the possible mechanism whereby the leukopenic factor of exudates induces sharp transitory leukopenia.

EXPERIMENTS

After a basal white cell count had been made on blood of a dog (the blood obtained by nicking a superficial vessel of the ear lobe) whole exudate, the leukopenic factor or pyrexin was injected into the heart of the animal. The material was usually introduced either in the fluid state or after having been suspended in varying concentration in isotonic solution of sodium chloride. Within about a half hour the number of circulating leukocytes was found to have dropped impressively. After a short interval, when the number of white cells in the circulating blood was still low, i. e., when there was distinct leukopenia, the animal was killed. A careful postmortem examination was made. Representative samples of tissue from various organs were fixed in a 10 per cent solution of

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This communication represents paper no. 35 of a series entitled "Studies on Inflammation."

1. Menkin, V.: (*a*) Am. J. Path. **16**:13, 1940; (*b*) Arch. Path. **30**:363, 1940.
2. Menkin, V.: Arch. Path. **41**:50, 1946.
3. Menkin, V.: Arch. Path. **34**:28, 1945.

formaldehyde or in Zenker's solution in which solution of formaldehyde U. S.P. had been substituted for glacial acetic acid in the concentration of 5 per cent (Helly's modification). Some of the tissues were also fixed for staining of glycogen and fat. As controls, some animals were studied after an intravascular injection of either isotonic solution of sodium chloride or some inert material.

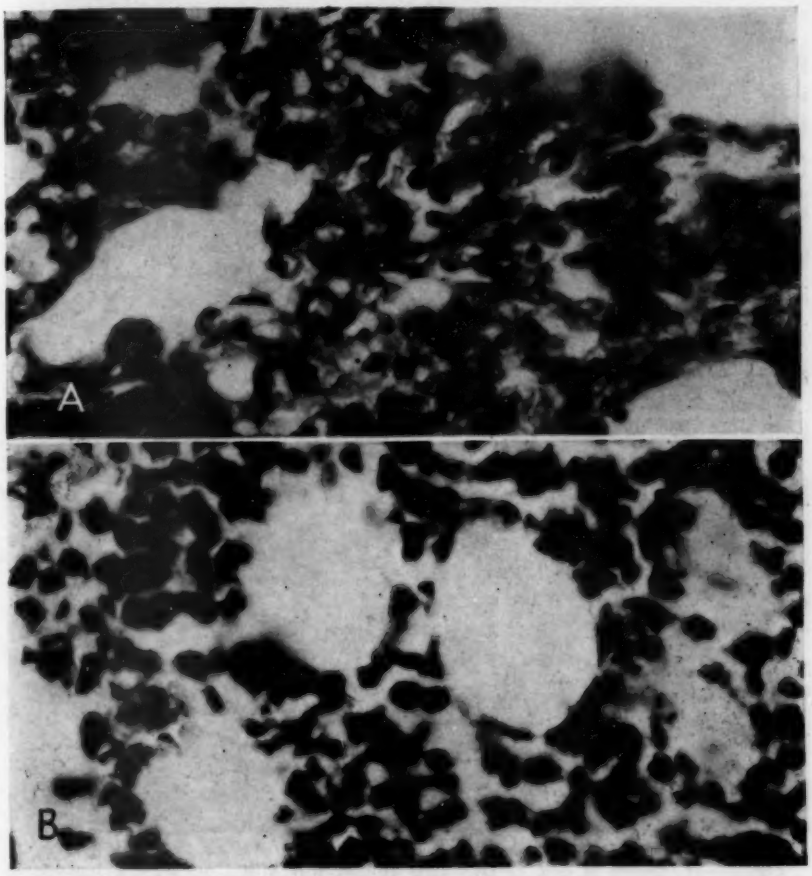

Fig. 1.—*A*, lung of dog 57-D. The animal was given 26 mg. of pyrexin by intracardiac injection and was killed during the leukopenic phase of the reaction. Note the thickening of the alveolar wall due to the trapping of leukocytes (× 695). *B*, bone marrow of the same dog. The hyperplastic appearance of the marrow is probably largely referable to trapping of leukocytes owing to the rapidity of their occurrence following injection of the material (× 695).

Microscopic sections revealed some interesting features. At the height of the leukopenic state many leukocytes were found trapped in various parts of numerous organs, including the alveolar walls of the lungs, the sinusoids of the liver and the pulp of the spleen. The bone marrow likewise apparently showed

retention of leukocytes.[4] Occasionally a few scattered leukocytes were found in the glomeruli of the kidney. Studies of differential blood smears indicated that the drop in the leukocyte count affected all types of leukocytes, i. e., the granulocytes as well as the monocytes. Some of the findings during the leukopenic phase are well illustrated by comparing a normal lung with that taken from an animal given an injection of the leukopenic factor (fig. 1 *A*). Figure 1 *B*

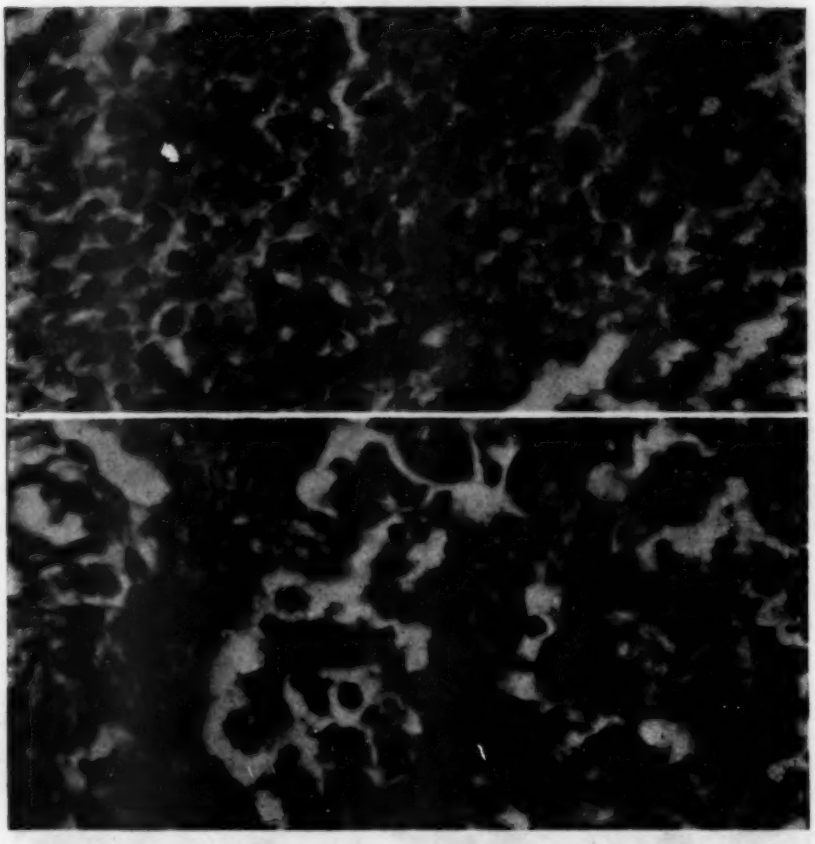

Fig. 2.—*A*, spleen of dog 57-D. This animal was killed during the leukopenic phase of the reaction following an injection of 26 mg. of pyrexin. The multitude of islets of leukocytes in the spleen may explain in part the mechanism of the acute splenic tumor accompanying numerous inflammatory processes (× 695). *B*, liver of the same dog. Leukocytes were caught in a number of sinusoids, as shown in the illustration (× 695).

reveals the condition of the bone marrow during the leukopenic phase. In figure 2 *A* is seen the trapping of leukocytes in the spleen. This splenic retention may perhaps be of help in elucidating further the mechanism of the acute splenic tumor accom-

4. It is conceivable in view of observations during the subsequent phase of leukocytosis that the picture of leukocytes being retained in the marrow is complicated by a superimposed hyperplastic response.

panying numerous inflammatory processes. The trapping of leukocytes in the sinusoids of the liver during the leukopenic phase is illustrated in figure 2 *B*. It is quite possible that the leukocytosis which develops after a period of acute leukopenia is referable in part to release of leukocytes that had been trapped in various tissues. The observations, however, do not preclude compensatory hyperplasia of the bone marrow, which may increase the subsequent number of circulating leukocytes. This phase is being studied further. No trapping was found in the cutaneous vessels of 1 animal studied with this in mind.

Finally, it is of some interest to note that the liver and often the myocardial tissue revealed extensive deposits of glycogen. This is reminiscent of a similar state of affairs observed after repeated intravascular injections of necrosin.[5] This phase of the work is also being studied further. A summary of data from the various experiments appears in the table.

Summary of Experimental Data

Dog	Dose of Pyrexin, Exudate or Leukopenic Factor Injected	Initial White Cell Count	White Cell Count Approximately at Time Animal Was Killed	Trapping of Leukocytes in Various Tissues			
				Lung	Liver	Spleen	Bone Marrow
57-D	26 mg. pyrexin	11,950	6,250	+	+	+	+
59-D	23.5 mg. pyrexin	13,600	3,650	+	+	+	+
69-D	6 cc. purulent exudate (p_H 5.2)	10,750	3,350	+	+	+	+
73-D	9 cc. leukopenic factor	12,050	4,400	+ (mild)	+	+	
77-D	28 cc. leukopenic factor	15,600	5,750	+	+	*	+
80-D	25 cc. leukopenic factor	16,350	4,200	+	+	+	0

+ This sign means that microscopic examination revealed trapping of leukocytes in the alveolar walls of the lung, in the sinusoids of the liver, in the splenic pulp (particularly around the malpighian corpuscles) and presumably in the marrow.

* Curiously enough, this animal showed an absence of the spleen.

COMMENT

The foregoing observations indicate that the leukopenia following the injection of an acid exudate, pyrexin or the leukopenic factor seems to be primarily referable to a trapping of leukocytes in the alveolar walls of the lung, in the sinusoids of the liver, in the pulp of the spleen and to a slight extent in the glomeruli of the kidney. There is apparently also trapping of leukocytes in the marrow, but studies made during the ensuing phase of leukocytosis suggest that besides trapping there may be compensatory hyperplasia in the marrow, probably to offset the abrupt leukopenic stage.

The findings are suggestive that the leukopenic factor of exudative material may be of significance in explaining the leukopenia which accompanies numerous inflammatory processes. It may be that the ultimate number of circulating leukocytes with inflammation depends in part on the resultant between two opposing factors in exudates. This is the leukopenic factor, on the one hand, which tends to depress the number of white cells, and the leukocytosis-promoting factor, on the other, which tends, in turn, to induce leukocytosis in the circulation.

5. Menkin, V.: Arch. Path., to be published.

The interplay of these two factors ultimately determines the actual number of circulating leukocytes.

Finally it should be pointed out that the intravascular injections of necrosin,[1b] of pyrexin and of the leukopenic factor are frequently accompanied by depositions of large amounts of glycogen in the hepatic cells and to some extent in cardiac muscle fibers. Whether in all these three fractions of exudates there is a common glycogen-producing factor remains to be determined. These studies are being pursued further.

SUMMARY

The leukopenia following the intravascular injection of an acid exudate, of pyrexin or of the leukopenic factor is accompanied by a trapping of apparently all types of leukocytes in the alveolar walls of the lungs, in the sinusoids of the liver, in the splenic pulp and perhaps in the marrow.

This trapping of leukocytes offers a reasonable explanation for the the mechanism of the leukopenia following the injection of the leukopenic factor.

The trapping of leukocytes in the pulp of the spleen may serve to explain in a reasonable way the primary mechanism of the acute splenic tumor accompanying numerous inflammatory processes.

Pyrexin, the leukopenic factor and necrosin seem to contain a common glycogen-inducing factor such that injection of any of these substances is followed by deposition of large quantities of glycogen in the hepatic cells and to some extent in the cardiac muscle fibers. This factor is being investigated further.

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EXPERIMENTAL ENDOCARDITIS OF DOGS

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IT IS commonly thought that bacterial endocarditis does not occur unless the cardiac valves have been previously damaged. Thus Kinsella [1] stated that "two factors are necessary in every case, a preexisting injury of the valve, and a recent infection which may invade the blood stream." Willius [2] in a recent review commented that "an injured heart valve is a prerequisite for development of subacute bacterial endocarditis." Christian,[3] however, stated that in only about 90 per cent of patients with bacterial endocarditis is there reason to believe that a previous injury of the heart valve existed. Christian's figures are more in accord with our own experience.

The earliest instance of experimental endocarditis produced without previous trauma of the cardiac valves was reported by Dreschfeld [4] in 1887. Since that time numerous investigators, using the technic of intravenous injection of organisms, have reported widely varying results. This disagreement persists in even the more recent literature.

Rosenow [5] produced endocarditis in normal rabbits by intravenous injections of streptococci isolated from the blood of patients with endocarditis.

MacNeal, Spence and Wasseen,[6] using rabbits and a strain of Streptococcus viridans isolated from a patient with subacute bacterial endocarditis, noted that typical vegetative lesions developed in 27 of 57 animals. Kinsella and Muether [7] were unable to produce endocarditis in dogs by injecting streptococci; however, after the valves had been

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1. Kinsella, R. A., in Cecil, R. L.: A Textbook of Medicine, ed. 6, Philadelphia, W. B. Saunders Company, 1944, p. 1074.

2. Willius, F. A.: Proc. Staff Meet., Mayo Clin. **19**:431, 1944.

3. Christian, H. A., in Osler, W.: Principles and Practice of Medicine, ed. 15, edited by H. A. Christian, New York, D. Appleton-Century Company, Inc., 1944, p. 1036.

4. Dreschfeld: Brit. M. J. **2**:887, 1887.

5. Rosenow, E. C.: J. Infect. Dis. **11**:210, 1912.

6. MacNeal, W. J.; Spence, M. J., and Wasseen, M.: Am. J. Path. **15**:695, 1939.

7. Kinsella, R. A., and Muether, R. O.: Arch. Int. Med. **62**:247, 1938.

injured mechanically, endocarditis could be produced by intravenous and even oral administration of the organisms.

Loewe, Rosenblatt and Lederer,[8] using five strains of Str. viridans, induced endocarditis in 35 per cent of a series of rabbits. The incidence was increased by the selection of potent strains, whose virulence was enhanced by growth on enriched mediums and passage through mice.

Blahd, Frank and Saphir[9] observed bacterial endocarditis in 40 per cent of 25 dogs whose heart valves were not previously injured, following one or more injections of streptococci isolated from the lung of a dog dying of pneumonia. They described the organisms as beta hemolytic streptococci. The authors suggested that the reason for their success, in contrast to the many failures previously reported in dogs, was that the strain of organisms employed was isolated from another dog, whereas other investigators had used bacteria isolated from human sources. Since these organisms were considered "substandard", their virulence was intensified by passing them through dogs not included in the series. Recently Loewe, Plummer, Niven and Sherman[10] have attempted to show that only a particular type of Str. viridans produces bacterial endocarditis.

METHOD OF STUDY

It has been our purpose to study this problem further and particularly to evaluate the importance of the type of organism, its virulence and the dosage in determining the incidence of endocarditis in dogs. A total of 99 animals were used, divided into two groups. In one group of 26 a planned effort was made to induce endocarditis. The other group, 73 animals, was given doses carefully regulated in an attempt to prevent the occurrence of endocarditis. In general, injections were given four times a week intravenously. The organisms were cultured in a broth of 0.3 per cent meat extract, 0.5 per cent salt and 1.0 per cent peptone with 0.2 cc. sterile dog blood added to 150 cc. of broth. Cultures were grown for twenty-four, forty-eight or seventy-two hours, depending on the rate of growth. No attempt was made to single out virulent organisms by testing of animals or to increase their virulence by special cultural methods. All organisms were isolated from routine clinical cultures, that is, from the noses, the throats,

TABLE 1.—*Incidence of Endocarditis Following Intravenous Injections of Bacteria*

	Dogs	Number with Endocarditis	Percentage with Endocarditis
Group 1	26	16	61.5
Group 2	73	10	13.7
Total	99	26	26.2

8. Loewe, L.; Rosenblatt, P., and Lederer, M.: Am. J. Path. **20**:89, 1944.

9. Blahd, M.; Frank, I., and Saphir, O.: Arch. Path. **27**:424, 1939.

10. Loewe, L.; Plummer, N.; Niven, C. F., and Sherman, J. M.: J. A. M. A. **130**:257, 1946.

the urine and the blood of normal patients and of patients with hypertension, scarlet fever, erysipelas and other diseases.

In group 1 strains of Str. viridans and of beta hemolytic streptococcus from thirteen different sources were used. In group 2 hemolytic and green-forming streptococci, diphtheria bacilli, staphylococci, colon bacilli, pneumococci and Bacillus mucosus were employed in addition to a number of those used in group 1. The organisms were obtained from forty-six sources.

Nineteen animals of group 1 were initially given large doses, varying from 50 to 100 cc. of broth; the remaining dogs received small doses, from 10 cc. to 50 cc. Fifty-one dogs of group 2 were initially given small doses, and 22 received large doses.

TABLE 2.—*Data on Dogs with Endocarditis*

Dog	Organism	Source	Time of Death	Location
1	Beta hemolytic streptococcus	Erysipelas	21 months	Aortic valve
2	Beta hemolytic streptococcus	Erysipelas	11 months	Mitral valve
3	Beta hemolytic streptococcus	Scarlet fever	16 months	Mitral valve
4	Beta hemolytic streptococcus	Pneumonia	3 months	Aortic valve
5	Beta hemolytic streptococcus	Pneumonia	6 days	Mitral valve
6	Beta hemolytic streptococcus	Pneumonia	6 days	Aortic and mitral valves
7	Beta hemolytic streptococcus	Pneumonia	25 days	Mitral valve
8	Beta hemolytic streptococcus	Pneumonia	11 days	Mitral valve
9	Beta hemolytic streptococcus	Pneumonia	17 days	Mitral valve
10	Beta hemolytic streptococcus	Pneumonia	7 months	Mitral and aortic valves
11	Beta hemolytic streptococcus	Scarlet fever	22 months	Mitral and aortic valves
12	Str. viridans	Culture of material from nose	35 months	Mitral and aortic valves
13	Str. viridans	Subacute bacterial endocarditis	5 months	Aortic valve
14	Str. viridans	Subacute bacterial endocarditis	7+ months	Aortic valve
15	Str. viridans	Culture of material from nose	12 days	Mitral valve
16	Str. viridans	Blood	1 month	Mitral valve
17	Str. viridans	Urine	2 months	Mitral valve
18	Str. viridans	Urine	12 months	Mitral valve
19	Str. viridans	Urine	27½ months	Mitral valve
20	Str. viridans	Urine	2 months	Mitral valve
21	Beta hemolytic streptococcus	Throat	33 months	Mitral valve
22	Beta hemolytic streptococcus	Pneumonia	82½ months	Mitral valve
23	Beta hemolytic streptococcus	Tonsil	15½ months	Mitral valve
24	Str. viridans	Tooth	5 months	Mitral valve
25	Beta hemolytic streptococcus	Throat	71½ months	Mitral valve
26	Str. viridans	Urine	24 months	Mitral valve

Group 2 was made up of animals which were used to produce experimental hypertension. This work has recently been reported.[11] In this series, the frequency with which endocarditis was obtained in the first series was kept in mind, and an effort was made to avoid endocarditis by keeping the doses low and discontinuing the injections for a time when untoward effects appeared, such as loss of weight, anorexia and fever.

RESULTS

Bacterial endocarditis developed in 26 of the 99 dogs. The organisms used were of 44 strains. Of these, 19 caused endocarditis in 1 or more instances. These figures are far more revealing, however, when broken down into two groups: In group 1 16 dogs showed autopsy-proved bacterial endocarditis. In 10 of the 73 animals of group 2 endo-

11. Dick, G. F.: Arch. Path. **39**:81, 1945.

carditis developed despite every effort made to prevent such an occurrence.

No strains other than those of Str. viridans and beta hemolytic streptococcus induced endocarditis. (These two organisms constituted more than 84 per cent of the strains used.) In 15 cases, beta hemolytic streptococcus was responsible, and in 11 Str. viridans. Since 46 animals were given the beta hemolytic and 37 the viridans type, it appears that the respective incidence is approximately the same. That this is a real and not an apparent distribution is borne out by the fact that the dosages of the organisms used in groups 1 and 2 were essentially the same.

TABLE 3.—*Incidence of Causative Organisms in Cases of Endocarditis*

	Dogs Inoculated	Number with Endocarditis	Percentage with Endocarditis
Beta hemolytic streptococcus	46	15	32
Str. viridans	37	11	30
Other organisms—diphtheria bacilli, B. mucosus, etc.	16	0	0

SUMMARY

A total of 99 dogs were given repeated intravenous injections of a variety of organisms isolated in routine cultures of all types. Forty-four different strains, chiefly strains of Str. viridans and beta hemolytic streptococcus, were used. No attempt was made to select strains or to increase their virulence. The dogs were placed in two groups: group 1, in which a definite attempt was made to induce endocarditis, and group 2, in which efforts were directed toward preventing its occurrence. In 61 per cent of group 1 endocarditis developed and, despite all precautions, 13 per cent of group 2 showed lesions of the same type. Positive results were obtained with the same frequency whether Str. viridans or beta hemolytic streptococcus was used. It has been our experience that the difficulty lies more in preventing occurrence of endocarditis than in producing it if streptococci are injected intravenously into dogs for any considerable period.

CONCLUSIONS

Contrary to existing opinion, bacterial endocarditis may be produced in dogs without previous injury of the cardiac valves.

It is not necessary to use streptococci from sites of endocarditis, as streptococci from a great variety of sources produce the disease in dogs.

While virulent strains produce endocarditis in shorter time and with fewer injections than do the less virulent strains, it is necessary only to continue the injections of the less virulent ones for a longer time and in increased doses to produce endocarditis regularly.

IN VITRO STUDIES ON THE PHYSIOLOGY OF CELLS

Interactions of Thymic Cells and an Oxidation-Reduction Indicator, 2,6-Dichlorophenolindophenol

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THE PHYSIOLOGIC reactions of cells have been studied in this laboratory by (1) the method of unstained cell counts, (2) the deferred histologic method and (3) the electrometric determination of the hydrogen ion concentrations of cellular suspensions.[1] These three methods are based on three distinct physiologic capacities of cells, namely: (1) the capacity of viable cells to resist staining with eosin; (2) the capacity of excised viable tissues to react histologically to reagents and (3) the capacity of cells to ferment glucose, with formation of acid. The methods have been found useful in studies on the reactions of cells to physical, chemical and biologic reagents such as oxygen, glucose, antiseptics, distilled water and roentgen rays.

A fourth physiologic capacity appears in the oxidation-reduction activity of cells. The present investigation is a preliminary study to determine whether this function can be used to develop another method of measuring the reactions of cells to reagents in vitro.

Oxidation-reduction activities have been studied extensively in suspensions of bacteria by Quastel,[2] Coulter and Isaacs,[3] Clifton[4] and Burrows and Jordan.[5] The oxidation-reduction properties of suspended animal cells have been investigated by Drew,[6] Voegtlin, Johnson and Dyer,[7] Cannan, Cohen and Clark[8] and Chambers, Beck and Green.[9]

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1. Schrek, R.: (*a*) Proc. Soc. Exper. Biol. & Med. **54**:283, 1943; (*b*) Arch. Path. **35**:857, 1943; (*c*) **37**:319, 1944; (*d*) Am. J. Path. **21**:1101, 1945; (*e*) Radiology **46**:395, 1946.

2. Quastel, J. H.: Biochem. J. **20**:166, 1926.

3. Coulter, C. B., and Isaacs, M. L.: J. Exper. Med. **49**:711, 1929.

4. Clifton, C. E.: J. Bact. **25**:495, 1933.

5. Burrows, W., and Jordan, E. O.: J. Infect. Dis. **58**:259, 1936.

6. Drew, A. H.: Brit. J. Exper. Path. **1**:115, 1920.

7. Voegtlin, C.; Johnson, J. M., and Dyer, H. A.: J. Pharmacol. & Exper. Therap. **24**: 305, 1924.

(Footnotes continued on next page)

The present communication reports observations on the reactions of thymic cells in suspension to an oxidation-reduction indicator, 2,6-dichlorophenolindophenol.

REDUCTION OF 2,6-DICHLOROPHENOLINDOPHENOL BY THYMIC CELLS

Effect of Mediums.—A suspension of thymic cells in phosphate–Ringer solution was prepared by the methods outline in a previous paper.[1e] To 0.2 cc. of this suspension was added 0.1 cc. of the reagent studied (such as serum or a solution of glucose or a phosphate–Ringer solution) and 0.2 cc. of 2,6-dichlorophenol-indophenol dissolved in phosphate–Ringer solution. The controls consisted of mixtures of comparable amounts of reagent and indicator without the addition of thymic cells. The test tubes were shaken horizontally one hundred and forty times per minute in a water bath maintained at 37 C. The mixtures were examined at intervals to determine the intensity of color. It was difficult to compare the experimental mixtures containing thymic cells and the control solutions without cells. Suspensions showing the indicator completely or partially

TABLE 1.—*Color Intensity of 2,6-Dichlorophenolindophenol Immediately and One Hour After Its Addition to a Mixture of Rabbit Thymic Cells and Reagent*

Final Concentration of Indicator	Intensity of Color with Reagent Added *				
	Phosphate-Ringer Solution	Rabbit Serum	Glucose M/6	Mannose M/6	Fructose M/6
1:5,000	5 - 5	4 - 2†	5 - 5	5 - 5	5 - 5
1:10,000	4 - 4	3 - 1†	4 - 3	4 - 4	4 - 4
1:20,000	3 - 3	2 - 0†	2 - 2	2 - 2	3 - 3
1:40,000	0 - 2	2 - 0†	0 - 0†	0 - 0†	0 - 2
1:80,000	0 - 1	1 - 0†	0 - 0†	0 - 0†	0 - 0†
1:160,000	0 - 0†	1 - 0†	0 - 0†	0 - 0†	0 - 0†
None	0 - 0	0 - 0	0 - 0	0 - 0	0 - 0

* The numbers indicate the intensity of the color of the indicator in the suspension: 5 representing maximal; 1, minimal; 0, no bluish color. The first number represents the intensity in color immediately after the addition of the indicator; the second number, after one hour of incubation at 37 C.

† The bluish color of the indicator was restored or intensified on the addition of potassium ferricyanide in a 0.1-molar solution.

decolorized in one hour were tested by the addition of 0.1 cc. of potassium ferricyanide (0.1 molar) to see whether the reduced dye could be reoxidized and the blue color restored.

A summary of several experiments on the reduction of the indicator by thymic cells is presented in table 1. It was observed that immediately after the dye in dilutions of 1:40,000 and 1:80,000 was added to the cells in a phosphate–Ringer solution the blue color of the indicator disappeared (table 1) but that it returned after a few minutes of incubation. The indicator in a dilution of 1:160,000 was immediately reduced by the thymic cells and remained reduced during the one hour incubation period. It seems, then, that washed thymic cells in phosphate–Ringer solution had the capacity of reducing 2,6-dichlorophenol-indophenol under aerobic conditions only when the solution of the dye was very dilute (1:160,000).

8. Cannan, R. K.; Cohen, B., and Clark, W. M., in Studies on Oxidation-Reduction, Hygienic Laboratory Bulletin no. 151, United States Public Health Service, 1928, p. 306.

9. Chambers, R.; Beck, L. V., and Green, D. E.: J. Exper. Biol. **10**:142, 1933.

Table 1 shows that in the presence of a small amount of homologous serum the thymic cells of the rabbit had decolorized completely a 1:20,000 solution of dye after one hour's incubation. Thymic cells of the rat in the presence of rat serum decolorized the dye to the same degree as the cells of the rabbit. It would seem, then, that thymic cells readily reduced 2,6-dichlorophenolindophenol even when the concentration was fairly high, in the presence of a small amount of homologous serum.

Mixtures of a suspension of thymic cells and a solution of glucose or mannose completely reduced the indicator in concentrations of 1:40,000 (table 1). The addition of fructose increased to a slight extent the capacity of thymic cells to reduce the indicator. In a previous work it had been shown that thymic cells can ferment glucose and mannose and possibly galactose but not other sugars.[1c] It would seem from these experiments that the capacity of thymic cells to reduce 2,6-dichlorophenolindophenol is increased by the addition of glucose, mannose or, to a lesser extent, fructose.

Effect of Solution of Formaldehyde U. S. P.—Several experiments were performed to determine whether the degree to which thymic cells reduce the indicator in the presence of serum could be affected by the addition of a toxic reagent such as formaldehyde. Solution of formaldehyde U. S. P. was diluted with phosphate–Ringer solution, and 0.1 cc. of each dilution was mixed with 0.2 cc. of thymic cell suspension, 0.1 cc. of rabbit serum and 0.2 cc. of a dilution of the indicator. The mixtures were incubated and examined for color periodically. The results of these experiments are summarized in table 2. It is seen from table 2 that when the concentration of solution of formaldehyde U. S. P. was very low, 1:7,680, the reagent had no perceptible effect on the capacity of the cells to reduce the dye in the presence of serum. A greater concentration of solution of formaldehyde U. S. P., 1:1,920, permitted rapid reduction of the indicator, but after four hours of incubation the bluish color of the indicator was restored spontaneously. This concentration of solution of formaldehyde U. S. P. had, then, a delayed effect on the capacity of the cells to reduce the indicator. With a still higher concentration of the reagent, 1:60, the decolorization of the indicator was inhibited. There is, then, an immediate and a delayed effect of formaldehyde on the cellular reduction of 2,6-dichlorophenolindophenol. With lower concentrations of the reagent, only the delayed response, namely reoxidation of the indicator, was observed. With higher concentrations of formaldehyde the immediate response, an inhibition of the reduction of the indicator, was also seen.

In one experiment the cellular suspension was diluted with an equal amount of phosphate–Ringer solution to reduce the number of cells. With the original, undiluted suspension, solution of formaldehyde U. S. P. diluted 1:60 inhibited the reduction of the indicator (table 2). With the diluted cellular suspension a lower concentration of solution of formaldehyde U. S. P., 1:120, sufficed to inhibit the complete reduction of the indicator. Evidently, the amount of solution of formaldehyde U. S. P. needed to inhibit the decolorization of the indicator varied directly with the number of cells in the suspension.

It is seen in table 2 that with a low concentration of the indicator, 1:40,000, there was required a fairly high concentration of solution of formaldehyde U. S. P., 1:60, to inhibit the reducing action of the thymic cells. With a higher concentration of the dye, 1:20,000, a lesser concentration of solution of formaldehyde U. S. P., 1:240, sufficed to inhibit the reduction of the indicator. The higher

the concentration of the dye, the lesser was the concentration of solution of formaldehyde U. S. P. required to inhibit reduction. It may be concluded that the amount of solution of formaldehyde U. S. P. required for inhibition of reduction varied directly with the number of cells and varied inversely with the concentration of the indicator.

One would assume that the observed inhibiting action of solution of formaldehyde U. S. P. on the reduction of the indicator may be due to a cytocidal action of the reagent on the thymic cells. This hypothesis was tested by the addition of eosin in Tyrode's solution in order to stain the dead cells. It was found, however, that all the cells were unstained or showed only a minimal amount of staining. Further experiments revealed that formaldehyde interfered with the eosin staining of cells known to be dead. The cytocidal action of formaldehyde cannot be measured by the method of counting eosin-resistant cells.

TABLE 2.—*Effect of Solution of Formaldehyde U. S. P. on the Capacity of Rabbit Thymic Cells to Reduce 2,6-Dichlorophenolindophenol in the Presence of Rabbit Serum*

	Intensity of Color of Indicator Incubated with Cells, Serum and Solution of Formaldehyde U. S. P.*								
Concentration of indicator	1:20,000			1:40,000					
Number of cells per millimicroliter	70			70			35		
Minutes of incubation	20	60	240	20	60	240	20	60	240
Final concentration of solution of formaldehyde, U. S. P.									
1:60	3	3	3	1	2	2	2	2	2
1:120	3	3	3	0	0	2	1	1	2
1:240	2	3	3	0	0	2	0	0	2
1:480	0	3	3	0	0	2	0	0	2
1:960	0	3	3	0	0	2			
1:1,920	0	0	2	0	0	1			
1:3,840	0	0	1	0	0	0			
1:7,680	0	0	0	0	0	0			
None	0	0	0	0	0	0	0	0	0

* The numbers indicate the intensity of the color of the indicator, as explained in table 1.

EFFECT OF 2,6-DICHLOROPHENOLINDOPHENOL ON GLYCOLYSIS

A suspension of rat thymic cells was incubated for one and four hours with a solution of glucose (M/30) and the oxidation-reduction indicator. The hydrogen ion concentrations of the mixtures were then determined by means of an electrometer equipped with a glass electrode and microchamber. Similar mixtures were incubated anaerobically in capillary glass tubes sealed with rubber tubing. The findings of one experiment are presented in table 3.

The p_H of a control thymic cell suspension incubated aerobically for one hour without the indicator was 7.35 in the absence of glucose and 6.98 in its presence. Evidently the amount of acid produced was only slight on aerobic incubation; i. e., aerobic glycolysis of thymic cells is low.

The effect of 2,6-dichlorophenolindophenol on aerobic glycolysis depended on the concentration of the dye. The p_H of the cell suspension–glucose solution mixture after one hour of incubation was decreased by moderate concentrations of the indicator (6.98 in the absence and 6.40 in the presence of a 1:40,000 concentration) but was not affected by low (1:160,000) or high (1:5,000) concen-

trations of the indicator. Apparently aerobic glycolysis is stimulated by moderate amounts but is not affected by low or excessive amounts of the indicator.

Under anaerobic conditions, in the absence of the indicator the p_H of the suspensions incubated for one hour was 7.08 in the absence and 6.03 in the presence of glucose. Anaerobic glycolysis is, then, quite marked. The anaerobic production of acid was slightly increased (p_H 5.84) by moderate concentrations of the indicator (1:40,000) and was markedly inhibited (p_H 6.95) by high concentrations (1:5,000).

It appears from these studies that glycolysis is high under anaerobic conditions or in the presence of a moderate concentration of the indicator. Glycolysis is low under aerobic conditions or in the presence of a high concentration of the indicator.

TOXICITY OF 2,6-DICHLOROPHENOLINDOPHENOL FOR THYMIC CELLS

Susceptibility of Rat and Rabbit Thymic Cells in Phosphate–Ringer Solution.—The toxicity of the dye for thymic cells of the rabbit was studied by the method of unstained cell counts.[1b] Mixtures of cells, reagent and dye were incubated

TABLE 3.—*Effect of 2,6-Dichlorophenolindophenol on the Hydrogen Ion Concentration of Suspensions of Rat Thymic Cells Incubated with Glucose Under Aerobic and Anaerobic Conditions*

Final Concentration of Indicator	p_H of Mixture		
	One Hour of Incubation		Four Hours of Incubation: Aerobic Conditions
	Aerobic Conditions	Anaerobic Conditions	
1:5,000	7.14 (5)*	6.95 (3)	7.10 (5)
1:10,000	6.82 (4)	6.84 (1)	6.84 (4)
1:20,000	6.65 (3)	6.49 (0)	6.24 (3)
1:40,000	6.40 (1)	5.87 (0)	5.62 (1)
1:80,000	6.81 (0)	5.84 (0)	6.78 (0)
1:160,000	6.90 (0)	5.95 (0)	6.96 (0)
1:320,000	6.95 (0)	5.97 (0)	7.02 (0)
None	6.98 (0)	6.03 (0)	7.06 (0)

* The numbers in parentheses indicate the intensity of color of the indicator, as explained in table 1.

at 37 C., and eosin, 1:1,000 in Tyrode's solution, was then added. A drop of the resulting mixture was placed in a hemocytometer. The unstained cells, the eosin-stained cells and the red blood cells were counted. For reasons presented in a previous paper [1b] the unstained cells were assumed to be viable and the stained cells were considered dead.

Table 4 shows the effect of the indicator on the number of unstained thymic cells in various mediums. In the first experiment to be considered, the thymic cells were obtained from a rabbit, and the medium consisted of phosphate–Ringer solution. Incubation had no appreciable effect on the number of unstained thymic cells in phosphate–Ringer solution without indicator (92.8 cells per millimicroliter before and 94.8 cells after an incubation period of two hours). Addition of the dye in 1:160,000 dilution caused a significant decrease in the number of unstained cells (from 92.8 to 40.4). A higher concentration of dye, 1:40,000, caused almost complete disappearance of the cells resistant to eosin (only 0.2 unstained cell per millimicroliter left). In this and in many other experiments it was definite that 2,6-dichlorophenolindophenol was extremely cytocidal to rabbit thymic cells in the presence of phosphate–Ringer solution.

A similar experiment was conducted with thymic cells of the rat. A 1:10,000 concentration of the dye failed to cause any definite decrease in the number of unstained cells (62.4 unstained cells per millimicroliter before and 51.6 after incubation, table 6). A concentration of 1:5,000 caused only a moderate decrease to 25.2 unstained cells. This is in sharp contrast to the experiment with the cells of the rabbit, in which a dilution of 1:80,000 was extremely toxic and reduced the number of unstained cells from 92.8 to 7.0. The 2,6-dichlorophenol-indophenol was markedly cytocidal to rabbit thymic cells in phosphate–Ringer solution but had only a minimal lethal action on the cells of the rat.

The question arises whether the toxic action of the indicator on rabbit thymic cells was due to light which had been absorbed and activated by the dye. To eliminate the action of light, the suspension and the dye were pipetted into test tubes which had been blackened on the outside by immersion in black enamel paint. The layer of paint was sufficiently heavy so that it was not possible to see the fluid in the test tubes even with bright illumination. The reagents were not protected from light before mixing. The mixtures of thymic cells and dye were

TABLE 4.—*Toxicity of 2,6-Dichlorophenolindophenol for Thymic Cells in Various Mediums*

	Number of Unstained Cells per Millimicroliter After Incubation of Dye, Thymic Cells and Reagent for Two Hours					
	Rat Thymic Cells:	Rabbit Thymic Cells				
Final Concentration of Dye	Phosphate-Ringer Solution	Phosphate-Ringer Solution	Rabbit Serum	Glucose M/6	Mannose M/6	Fructose M/6
1:5,000	25.2 (5)*	0.3 (5)	4.0 (5)	.....	0.3 (5)	0.0 (5)
1:10,000	51.6 (5)	0.0 (4)	67.2 (3)	8.4 (3)	2.2 (4)	0.3 (4)
1:20,000	52.0 (4)	0.2 (3)	97.6 (1)	56.4 (0)	63.2 (1)	0.5 (3)
1:40,000	59.2 (3)	0.2 (2)	96.4 (0)	87.6 (0)	76.8 (0)	1.3 (2)
1:80,000	53.6 (2)	7.0 (1)	.....	80.4 (0)	97.6 (0)	90.0 (0)
1:160,000	.....	40.4 (0)	.....	.....	88.4 (0)	76.8 (0)
None	52.4	94.8	94.0	90.0	94.4	97.6
Before incubation	62.4	92.8	92.8	108.2	93.6	93.6

* The numbers in parentheses indicate the intensity of color of the indicator, as explained in table 1.

incubated in the blackened test tubes and in ordinary tubes. After two hours of incubation, eosin was added to the mixtures. A drop was placed in the hemocytometer, and the cells were immediately examined and counted. It was found that there was no appreciable difference in the unstained cell counts of the mixtures in the painted and in ordinary test tubes. Apparently, the toxic action of 2,6-dichlorophenolindophenol on thymic cells of the rabbit was not due to the dynamic action of light.

Rate of Toxic Reaction.—The rate of the cytocidal action of the dye was determined as follows: A solution of the indicator was sterilized by passing it through a Seitz filter. A sterile suspension of thymic cells of the rabbit was incubated with the indicator at 37.0 C. Unstained cell counts were made periodically. The data were represented graphically on logarithmic-probability paper, and the 50 and 10 per cent survival periods were estimated from the graphs. It was observed that 90 per cent of the cells died in 0.7 hour when incubated with indicator 1:20,000, in 3.1 hours with indicator 1:80,000, in 7.6 hours with indicator 1:160,000 and in 42.5 hours in the absence of indicator. It would seem that the toxic action of the higher concentrations of the indicator was rapid.

Even very low concentrations had a perceptible toxic action on thymic cells of the rabbit.

Effect of Serum.—The medium of the experiments on the toxic action of the indicator was a phosphate–Ringer solution. The effect of the addition of serum is shown in table 4. It was observed that the indicator even in a concentration of 1:20,000 failed to cause any appreciable change in the unstained cell count in the presence of serum (97.6 and 94.0 cells per millimicroliter with and without dye, respectively). Evidently, rabbit serum inhibited the cytocidal action of 2,6-dichlorophenolindophenol on thymic cells of the rabbit.

Further experiments were done to determine how much serum was needed to neutralize the toxic effect of the dye. It is seen from table 5 that with a dye concentration of 1:40,000 it took 0.025 cc. of serum to neutralize the toxic effect; with a concentration of 1:20,000, 0.05 cc. of serum, and with a concentration of 1:10,000, 0.1 cc. In this roughly quantitative experiment it appeared that the greater the amount of dye used the greater was the amount of serum required to neutralize the toxicity. The volume of serum needed was approximately proportional to the concentration of the dye.

TABLE 5.—*Effect of Serum on the Cytocidal Action of 2,6-Dichlorophenolindophenol*

	Number of Unstained Cells per Millimicroliter After Two Hours of Incubation				
Concentration of indicator.....	1:10,000	1:20,000	1:40,000	1:40,000	1:40,000
Dilution of suspension of thymic cells of rabbit.....	Undiluted	Undiluted	Undiluted	Diluted 1:2	Diluted 1:4
Cc. of serum added					
0.1.....	82.4 (0)*	69.2 (0)	72.4 (0)	37.6 (1)	17.6 (2)
0.05.....	1.8 (3)	63.6 (1)	78.0 (0)	40.4 (1)	14.0 (2)
0.025.....	0.5 (4)	0.3 (2)	85.6 (0)	32.0 (2)	0.3 (3)
0.0125.....	0.0 (4)	0.0 (4)	1.6 (2)	0.9 (3)	0.0 (3)
0.0062.....	0.0 (4)	0.0 (4)	0.7 (2)	1.4 (3)	0.0 (4)
0.0031.....	0.0 (4)	0.2 (4)	0.6 (2)	0.2 (3)	0.0 (4)
None.....	0.3 (4)	0.0 (4)	0.6 (2)	0.0 (3)	1.4 (4)

* The numbers in parentheses indicate the intensity of color of the indicator, as explained in table 1.

In another experiment summarized in table 5, the dye was kept constant, and the serum and the cells were the variable factors. The undiluted cellular suspension required approximately 0.025 cc. of serum to protect the thymic cells against the toxic action of dye in a 1:40,000 dilution. The suspension diluted 1:4 necessitated only a slightly larger amount of serum, 0.05 cc., for protection against the same amount of dye. In this and in other experiments it appeared that when the concentration of the dye was kept constant a lesser number of cells required a slightly larger amount of serum for protection against the cytocidal action of the dye. The amount of serum required then varied inversely with the number of cells in the suspension and was directly proportional to the concentration of the dye.

Effect of Glucose.—In experiments similar to those just described, glucose was substituted for serum. According to table 4, this reagent protected thymic cells of the rabbit against the toxic action of the indicator in a dilution of 1:40,000 (87.6 and 90.0 cells per millimicroliter, with and without dye). The same concentration of dye caused an almost complete disappearance of eosin-resistant cells in the absence of glucose (0.2 and 94.8 unstained cells, with and without dye). It may be concluded, then, that glucose caused the reduction of the indicator and

protected the thymic cells of the rabbit against the cytocidal action of 2,6-dichlorophenolindophenol.

An experiment was performed to determine how much dextrose is required to neutralize the toxic effect of the dye. With a concentration of indicator of 1:40,000 and a M/7290 solution of glucose, the number of unstained cells dropped from 70.0 to 13.0 cells per millimicroliter (table 6). With the dye in a 1:20,000 dilution, a M/810 solution of glucose was required to protect 12.5 cells per millimicroliter. When the concentration of the indicator was 1:10,000, the maximum amount of glucose used (M/30) protected only 7.6 cells per millimicroliter. It would seem, then, that a moderate increase in the amount of indicator added necessitated a considerable increase in the glucose required for the protection of the cells. With the serum, on the other hand, a moderate increase in the dye required only a proportional increase in the amount of serum needed.

TABLE 6.—*Effect of Glucose on the Cytocidal Action of 2,6-Dichlorophenolindophenol*

	Number of Unstained Cells per Millimicroliter After Two Hours of Incubation				
Concentration of indicator.....	1:10,000	1:20,000	1:40,000	1:40,000	1:40,000
Number of unstained thymic cells of rabbit before incubation.....	70.0	70.0	70.0	23.6	15.2
Concentration of glucose, molarity					
1/30.....	7.6 (4)*	46.4 (1)	70.4 (0)	24.8 (0)	5.2 (1)
1/90.....	6.4 (4)	45.2 (1)	64.8 (0)	25.6 (0)	2.8 (1)
1/270.....	4.0 (4)	28.8 (2)	65.2 (0)	20.8 (0)	3.1 (1)
1/810.....	2.6 (4)	12.5 (2)	55.6 (0)	8.0 (1)	3.1 (2)
1/2,430.....	0.7 (4)	0.4 (3)	10.8 (1)	2.7 (2)	.....
1/7,290.....	.....	0.5 (3)	13.0 (1)	.....	.....
1/21,870.....	.....	.....	2.6 (2)	.....	.....
None.....	0.6 (5)	0.0 (3)	1.2 (2)	0.7 (2)	0.8 (2)

* The numbers in parentheses indicate the intensity of color of the indicator, as explained in table 1.

The effect of varying the number of cells in suspension is also shown in table 6. When the mixtures contained the undiluted cellular suspension (70 cells per millimicroliter), a M/810 solution of dextrose was required for protection of the cells. With the suspension diluted approximately 1:4 (15.2 cells per millimicroliter), even a M/30 solution of glucose protected only one third of the cells. Evidently, then, the higher the dilution of cells, the greater was the amount of glucose required to protect the cells against the toxic effect of 2,6-dichlorophenolindophenol.

COMMENT

The objectives of the present and previous investigations on cellular physiology are (1) to develop methods for studying the physiology of cells and (2) to determine differences in the physiologic reactions of the various types of cells. In the present phase of this work the dye 2,6-dichlorophenolindophenol has been studied to determine its utility as an oxidation-reduction indicator in cellular suspensions.

The first problem that arises in such a study is the determination of the ability of the cells to reduce the indicator. It has been shown by

Gibbs, Cohen and Cannan [10] that 2,6-dichlorophenolindophenol can be readily reduced. They made potentiometric measurements which proved that mixtures of equal amounts of the oxidized and the reduced indicator had the high potential of 0.216 at p_H 7.0. Voegtlin, Johnson and Dyer [7] observed that a related dye, 2,6-dibromophenolindophenol, was rapidly reduced by suspensions of viable normal and viable cancerous tissues under anaerobic conditions but was not reduced by necrotic cancerous tissue. Cohen, Gibbs and Clark [11] reported 2,6-dibromophenolindophenol reduced by neutralized suspensions of macerated plant tissue even when a vigorous stream of air was passed through the suspension. Needham and Needham [12] injected this compound into the cytoplasm of marine eggs under aerobic conditions and observed that the indicator was reduced and became colorless. They also showed that the reduced dye could be reoxidized to its original color by potassium ferricyanide. Gibbs, Cohen and Cannan [10] believed it significant that all living cells are able to reduce 2,6-dibromophenolindophenol even in the presence of oxygen. In the present study, suspensions of washed thymic cells of the rat or the rabbit (100 cells per millimicroliter) in phosphate–Ringer solution reduced and kept reduced 2,6-dichlorophenolindophenol, 1:160,000, for many hours.

The second problem that arises is the capacity of the system to resist changes in the oxidation-reduction potential. This problem is analogous to the measurement of the buffering action of a solution at a given p_H. Cohen, Chambers and Reznikoff [13] have shown that amebas can decolorize 2,6-dichlorophenolindophenol on repeated injections but that ultimately the capacity of the cytoplasm to reduce is overwhelmed. In the present study the capacity of the suspended cells to reduce was measured by adding indicator in varying concentrations to a constant amount of cells. The maximum concentration in which the indicator was completely reduced by the suspended cells was used as a measure of the oxidation-reduction capacity of the cells. For example, washed thymic cells in phosphate–Ringer solution reduced the indicator when its concentration was 1:160,000 but not when its concentration was 1:80,000. In contrast, washed thymic cells plus serum reduced the dye completely even when the concentration was 1:20,000. It is

10. Gibbs, H. D.; Cohen, B., and Cannan, R. K., in Studies on Oxidation-Reduction, Hygienic Laboratory Bulletin no. 151, United States Public Health Service, 1928, p. 159.

11. Cohen, B.; Gibbs, H. D., and Clark, W. M., in Studies on Oxidation-Reduction, Hygienic Laboratory Bulletin no. 151, United States Public Health Service, 1928, p. 138.

12. Needham, J., and Needham, D. M.: Proc. Roy. Soc., London, s.B **99:** 173, 1926.

13. Cohen, B.; Chambers, R., and Reznikoff, P.: J. Physiol. **11:**585, 1928.

obvious, then, that the cells plus serum had a much greater reducing capacity than the cells in phosphate–Ringer solution.

A third problem in oxidation-reduction is to determine the effect of substrates on the reducing intensity and capacity of the suspended cells. An extensive amount of work has been done by Quastel [2] on a large number of substrates that enabled washed bacterial cells to reduce methylene blue under anaerobic conditions. No similar studies have been performed on animal cells. Chambers, Beck and Green,[9] however, observed that ethyl alcohol increased the rate at which the eggs of starfish reduced methylene blue. In the present study, glucose and serum were found to increase the capacity of suspended thymic cells to reduce 2,6-dichlorophenolindophenol. The concentration of glucose in the serum was not sufficient to account for the marked increase in the reducing capacity of the thymic cells. The use of 2,6-dichlorophenolindophenol suggested then that glucose and serum reacted with thymic cells.

Certain agents have an inhibiting instead of a stimulating effect on the reducing intensity or capacity of the cells. Chambers, Beck and Green [9] found that mercuric oxide in a 1 : 1,000,000 solution inhibited completely the anaerobic reduction of methylene blue in suspensions of the eggs of starfish. Lead carbonate 1 : 1,000,000 and ethyl carbamate in a 3 per cent solution retarded the rate of reduction. In the present study, solution of formaldehyde U. S. P. was found to have an immediate and a delayed inhibiting action on the reduction of 2,6-dichlorophenol-indophenol. Presumably these inhibitory substances acted by killing the cell, by inhibiting an enzymatic reaction or by combining with a substrate. It seemed that the toxicity of a reagent could be studied by the simple method of determining its inhibition of the power of suspended cells to reduce 2,6-dichlorophenolindophenol.

The use of indicators to measure oxidation-reduction potentials in cellular suspensions has several complications. As Cannan, Cohen and Clark [8] pointed out, it is important to determine whether the dye itself is toxic or cytocidal. Chambers, Cohen and Pollack [14] reported that 2,6-dichlorophenolindophenol can penetrate into the echinoderm ovum and that it is toxic. Voegtlin, Johnson and Dyer [7] studied the toxicity of 2,6-dibromophenolindophenol in the rat on intravenous injection but did not study its toxicity to the cells in suspension. The present study has shown that 2,6-dichlorophenolindophenol has a marked cytocidal action on washed rabbit thymic cells but not on rat thymic cells in phosphate–Ringer solution.

In a previous study it was observed that thymic cells of the rabbit, but not those of the rat, were rapidly killed on incubation at 45 C. in

14. Chambers, R.; Cohen, B., and Pollack, H.: J. Exper. Biol. 8:1, 1931.

the absence of glucose and air. That observation may be compared with the present finding that thymic cells of the rabbit, but not those of the rat, were rapidly killed by 2,6-dichlorophenolindophenol. Presumably both reactions are based on one common factor or deficiency in the thymic cells of the rabbit. What this factor may be would require further investigation.

A second complicating factor in the use of 2,6-dichlorophenolindophenol as an oxidation-reduction indicator is modification of metabolism of the cells by the indicator. Elliot [15] and Elliot and Baker [16] observed that this indicator produced almost complete inhibition of the respiration of tumor tissue in the absence of glucose but accelerated respiration in the presence of glucose. They also found that the indicator inhibited the respiration of kidney, brain, testis and chick embryo both in the presence and in the absence of glucose. The dye had no effect on the glycolysis of tumor. They do not state whether the indicator's inhibition of respiration is due to an inhibiting effect on cellular metabolism or to a lethal action on the cells.

In the present study the effect of the indicator on glycolysis was studied. It was shown that the dye in moderate concentrations stimulated production of acid in the presence of glucose and air. In higher concentrations, however, the dye inhibited glycolysis both under aerobic and under anaerobic conditions. The indicator 2,6-dichlorophenolindophenol has, then, an accelerating or an inhibiting effect depending on the concentration of the dye.

Two conclusions may be drawn: The oxidation-reduction indicator 2,6-dichlorophenolindophenol may be used to determine substances which have a stimulating or an inhibiting effect on cells. It is also useful as a reagent which affects the glycolysis of thymic cells and which has even in dilute solutions a cytocidal action on washed thymic cells of the rabbit but not on those of the rat. It can be used either as an oxidation-reduction indicator or as a reagent, depending on its concentration, on the time of incubation and on the presence of other substances.

SUMMARY AND CONCLUSIONS

Glucose, mannose and serum increased the capacity of thymic cells to reduce 2,6-dichlorophenolindophenol. Solution of formaldehyde U. S. P. caused an immediate or a delayed inhibition of their power to reduce the dye. Evidently, 2,6-dichlorophenolindophenol as an oxidation-reduction indicator is useful in determining the effects of reagents on cells.

15. Elliot, K. A. C.: Nature, London, **134**:254, 1934.
16. Elliot, K. A. C., and Baker, Z.: Biochem. J. **29**:2396, 1935.

By the method of unstained cell counts it was found that the indicator even in a dilution of 1:160,000 had a cytocidal effect on washed thymic cells of the rabbit but not on those of the rat. The cytocidal action was inhibited by serum, glucose and mannose.

According to electrometric determinations of hydrogen ion concentrations, the dye in moderate concentrations (1:40,000) caused a considerable increase in aerobic glycolysis and a slight increase in anaerobic glycolysis. In higher concentrations (1:5,000) the indicator inhibited both aerobic and anaerobic glycolysis.

It seems, then, that, in addition to being a useful oxidation-reduction indicator, 2,6-dichlorophenolindophenol is an interesting reagent which affects the metabolism of the cells and which shows a differential reaction between rabbit and rat thymic cells.

CEREBRAL CONCUSSION

Histochemical Demonstration of Nucleases in the Cerebrospinal Fluid

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IN SPECTROPHOTOMETRIC studies of the cerebrospinal fluid in ultraviolet rays, Spiegel-Adolf, Wycis and Spiegel [1] demonstrated, following cerebral concussion, the appearance of substances giving a selective absorption band with a peak at 265 millimicrons. This finding was interpreted as due to the entrance of nucleic acids or their derivatives. If the cerebrospinal fluid was left standing, the selective absorption became weaker and finally disappeared even though the punctate was kept under sterile conditions. It was suspected that this behavior was due to the appearance of enzymatic substances. In order to test this hypothesis, cerebrospinal fluids from normal persons as well as from patients who had sustained cerebral concussion were incubated at 37 C. with samples of nucleic acids of animal and of plant origin. Both types of nucleic acids when incubated with the concussion specimens showed a decrease of the selective absorption, while the samples incubated with normal cerebrospinal fluid or saline solution remained unchanged. These experiences seemed to confirm the assumption that enzymatic substances acting on nucleic acids or their derivatives, or both, appear in the cerebrospinal fluid after cerebral concussion.

Spectrophotometric demonstration of such enzymes requires knowledge of a special technic as well as a rather expensive apparatus which is beyond the reach of many routine laboratories. It seemed desirable, therefore, to develop a technic which the average laboratory can easily handle. For this practical reason, as well as from a theoretic point of view, it seemed of interest to study whether an effect similar to that observed in cerebrospinal fluid from patients who had undergone concussion could be observed when the test objects were not nucleic acids

This investigation was aided by a grant from the John and Mary R. Markle Foundation.

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1. Spiegel-Adolf, M.; Wycis, H. T., and Spiegel, E.: Federation Proc. **5**:156, 1946.

but nuclear substances within the cells of the central nervous system, in particular the Nissl bodies of the nerve cells. The principle of the method to be described consists in incubating the cerebrospinal fluid under study with paraffin sections of normal spinal cord and to stain these slides by means of the usual Nissl method (with methylene blue, thionine blue or cresyl violet).

In preliminary experiments the effect of normal cerebrospinal fluid on the anterior horn cells of the cat's spinal cord was studied. To our surprise, such cerebrospinal fluid produced tigrolysis in the anterior horn cells. The specimens of cerebrospinal fluid were used immediately after lumbar puncture and were kept under sterile conditions. Further experiments showed that even Ringer's solution when incubated for several hours at 37 C. with sections of cats' spinal cords was able to bring the Nissl bodies into solution. It occurred to us that old experiments of Held [2] had pointed to the importance of the acidity of the solution for the solubility of the Nissl bodies. Therefore, by adding buffer solutions to Ringer's solution or to the cerebrospinal fluid under study, the hydrogen ion concentration of the solution or fluid acting on the sections of spinal cord was varied systematically. It was found that solution or fluid with a p_H between 2.0 and 4.1 did not affect the Nissl bodies significantly when incubated at 37 C. for four to five hours. When solutions of a p_H of 4.6 or higher were used, the Nissl bodies of the anterior horn cells were much finer than normal. When the p_H of the solution was above 6.0, definite signs of solution of the Nissl bodies appeared. These experiments seemed to indicate that at the hydrogen ion concentration of the normal cerebrospinal fluid the Nissl bodies go into solution after several hours' incubation at 37 C. These experiences may also shed some light on the old question whether the Nissl bodies are preformed in the living cells (Bielschowsky [3]). Apparently, at the normal hydrogen ion concentrations of the body fluids or cell fluids the nucleoproteins of the nerve cells are kept in solution, and only when postmortem changes cause an accumulation of acids in the central nervous system are the nucleoproteins precipitated —and the results are the Nissl bodies. It proved important, therefore, to acidify the cerebrospinal fluid before it was incubated with the spinal cord slides. As a rule, a buffer solution with a p_H of 4.05 was added to the cerebrospinal fluid. Thus the following method was finally adopted:

The test objects are, as a rule, the spinal cords of cats. The cords are fixed in 70 per cent alcohol. The blocks are embedded in paraffin by the usual method,

2. Held, H.: Arch. f. Anat. u. Physiol. (Anat. Abt.), 1895, p. 396.

3. Bielschowsky, M.: Morphologie der Ganglienzellen, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1928, vol. 4, p. 8.

and paraffin sections 5 to 10 microns in thickness are cut. Two pairs of sections of the cat's cord are placed on a slide. After the sections are deparaffinized, each pair is surrounded by a square frame corresponding to the size of a cover glass of 18 sq. mm. Such slides may be kept in stock for testing specimens of cerebrospinal fluid as soon as they are brought from the operating room.[4] It has been found that 0.25 to 0.50 cc. of cerebrospinal fluid is sufficient for such a test. As a rule, 0.45 cc. of the fluid is mixed with 0.05 cc. of an acetate buffer[5] of p_H 4.05 in a sterile tuberculin syringe. About 0.2 to 0.25 cc. is necessary to fill the area within the paraffin square. After this amount of fluid has been deposited within the paraffin frame, it is covered by a clean cover glass, and the edges between the cover glass and the frame are sealed with paraffin so that they are air tight. It is important that the fluid cannot evaporate when the slide is put in the incubator and that no air bubbles are left within the sealed area, since changes in the concentration of the fluid may affect the nerve cells. Usually one pair of sections is covered with cerebrospinal fluid and the other with Ringer's solution, which is also acidified with the buffer solution in the same proportion, 9 to 1. The same procedure may be repeated on the second slide, or on the second slide only one square is covered with cerebrospinal fluid and the other square left without fluid. The slides are then put in an incubator at 37 C. for four hours.[6] At the end of this period the cover glasses and the paraffin frames are removed, and the slides are washed in distilled water and subjected to the routine staining with 0.1 per cent thionine solution. On differentiation of the slides with alcohol, it is important to check the progress of the differentiation under the microscope repeatedly. If the cerebrospinal fluid has produced a high degree of tigrolysis, the differentiation should not be too intense, so that the cytoplasm of the cells is still clearly visible. In such a case the control sections which were under the influence of Ringer's solution or were not subjected to any fluid at all are, of course, slightly overstained. In any case it is important to treat both pairs of sections, those under the influence of cerebrospinal fluid and the control sections, in exactly the same manner.

In figure 1 the parts lettered *a* show anterior horn cells of the spinal cord of a normal cat which were subjected to the cerebrospinal fluid of a patient with cerebral concussion. The interval between the trauma and the lumbar puncture was three days. A tigroid structure is hardly visible; the major part of the cytoplasm is only faintly stained, appearing homogeneous or slightly granulated, while its remaining part shows a more or less homogeneous, darkly stained mass. The peripheral part of the cell usually is more affected than the central part. Control sec-

4. As a rule, the specimens were studied within a few hours after tapping. Control experiments showed that specimens preserved in an ice box for a few days under sterile conditions can still be used for this enzyme test, while a specimen standing in the ice box for over a week may affect the anterior horn cells on incubation even if this specimen is taken from a patient with a normal central nervous system.

5. The buffer is prepared by mixing 15.0 cc. of molar acetic acid with 3.0 cc. of molar sodium acetate.

6. In some of our earlier experiments we kept the slides for four to five hours at 37 C. and for seventeen to nineteen hours at room temperature (23-25 C.).

tions (fig. 1 *b* and 1 *c*)—one kept in the incubator without the addition of any fluid and one subjected to the influence of Ringer's solution —are overstained for the reasons mentioned in the foregoing paragraph.

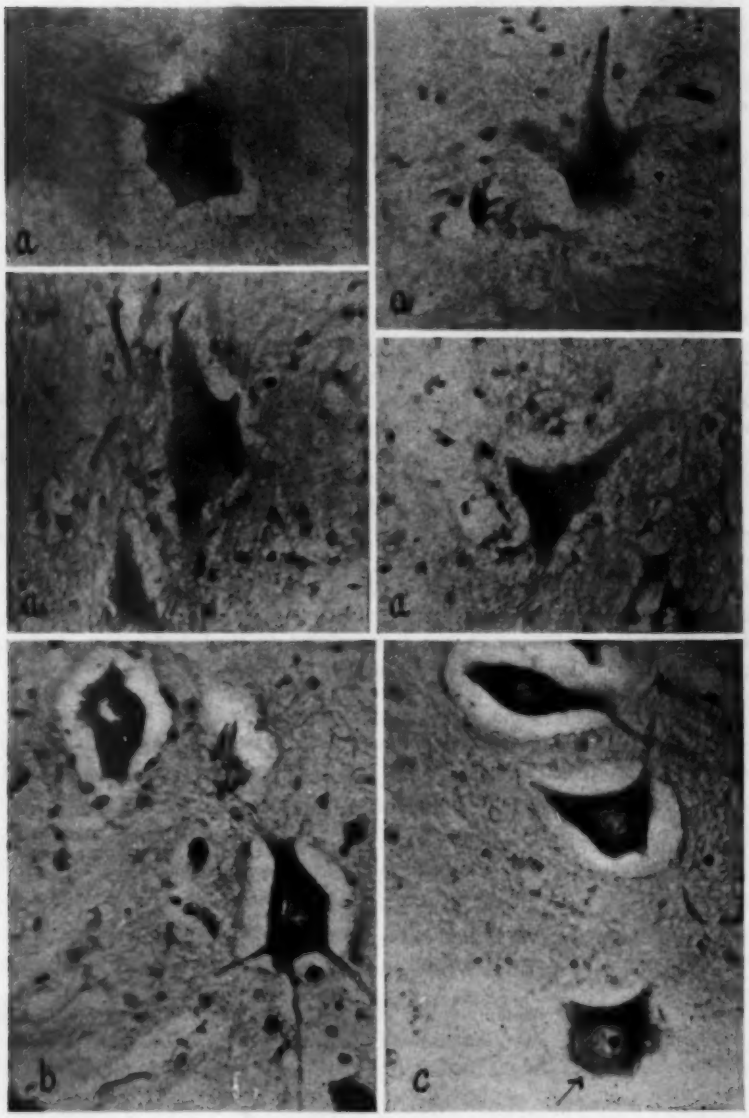

Fig. 1.—Anterior horn cells in sections from the spinal cord of a normal cat. The sections were stained with toluidine blue after incubation (*a*) with cerebrospinal fluid from patient W., who had sustained cerebral concussion, (*b*) without addition of any fluid and (*c*) with Ringer's solution. The interval between the cerebral trauma and the lumbar puncture was three days. For further details see the text.

But they show clearly that in the large majority of the cells the Nissl bodies are well preserved. Only occasionally (arrow in fig. 1 *c*) a cell shows an area in its periphery where the tigroid bodies are only faintly stained or are not demonstrable. Figure 2 *a* represents the effect of the cerebrospinal fluid from another patient with concussion, which was tapped twelve days after the cerebral trauma. The solution of the nucleoproteins is still more advanced, probably because the specimens were subjected to the influence of the fluid for a longer time (five hours at 37 C., seventeen hours at 25 C.). Practically all the anterior horn cells fail to show Nissl bodies; the cytoplasm represents a homogeneous mass, sometimes traversed by fissures; only the nucleolus may be demonstrable. In contradistinction the glia nuclei seem rather well preserved. The

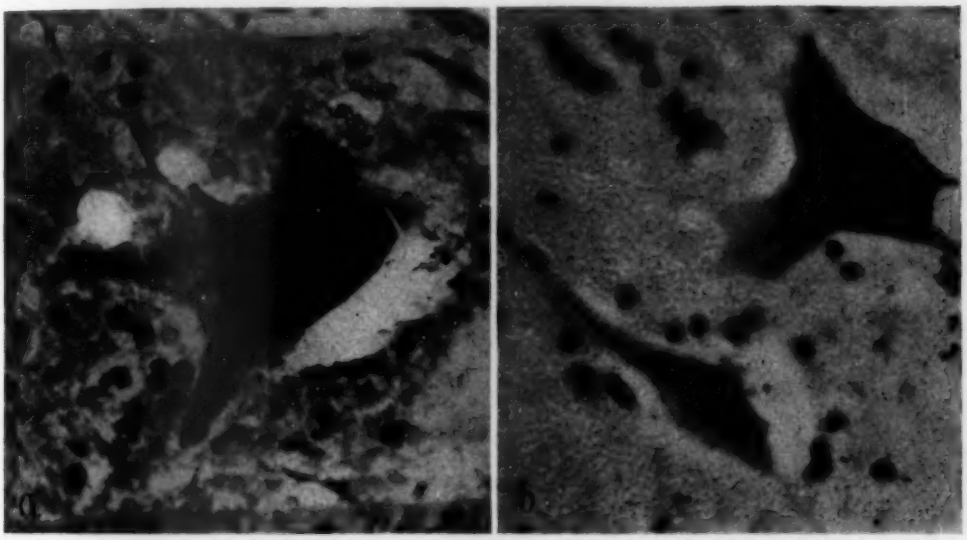

Fig. 2.—Anterior horn cells in sections from the spinal cord of a normal cat which were stained with cresyl violet after incubation with (*a*) cerebrospinal fluid from patient C., who had undergone cerebral concussion, and (*b*) Ringer's solution. The interval between the accident and the lumbar puncture was twelve days.

intercellular tissue is more darkly stained than that in the control sections, which were subjected to Ringer's solution (fig. 2 *b*), perhaps because the dissolved nucleoproteins or their cleavage products diffused into the pericellular tissue. It should be emphasized that the tigroid bodies of single cells may be affected also if buffered Ringer's solution or cerebrospinal fluid of patients with apparently normal central nervous system is incubated with sections of cats' cords. Therefore the conclusion that one deals with nucleases or similar enzymatic substances seems warranted only if the cerebrospinal fluid is able to produce tigrolysis in all or in the majority of the anterior horn cells studied.

While figures 1 and 2 represent cases in which the cerebrospinal fluid was tapped within a few days after the trauma, figure 3 shows the influence of a cerebrospinal fluid that was obtained one-half year after the accident that produced the cerebral concussion. This fluid was no longer able, on incubation, to affect the cells of the cat's anterior horn.

In a parallel study, these cerebrospinal fluids were studied by spectrophotometry in ultraviolet rays, and it was found that the fluids producing tigrolysis were able to decrease the characteristic absorption power of nucleic acids, while the fluids which left the anterior horn cells intact also failed to affect the nucleic acids. Thus the histochemical and the spectrophotometric method confirmed each other.

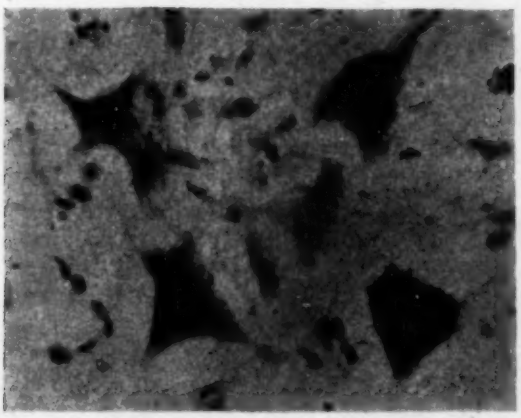

Fig. 3.—Anterior horn cells in a section from the spinal cord of a normal cat. This section was incubated with cerebrospinal fluid of patient D. (cerebral concussion) and then stained with toluidine blue. The interval between the accident and the lumbar puncture was one-half year.

The demonstration that the cerebrospinal fluid of patients with cerebral concussion contains substances able to produce solution or a breakdown of Nissl bodies seems of interest not only from a practical but also from a theoretic point of view. If such substances diffuse from the central nervous system into the cerebrospinal fluid, it seems not unreasonable to suspect that they play an important role in the genesis of the chromatolytic changes observed after concussion.[7]

7. The chemical nature of the enzymes taking part in the mechanism of tigrolysis remains to be studied. It seems probable that they are not only nucleases but also desaminases. The absorption band at 265 millimicrons found by spectrophotometric study of cerebrospinal fluid from patients who had undergone cerebral concussion can be caused not only by nucleic acids but also by some of their cleavage products, such as pyrimidine bases. It has been pointed out that the cerebrospinal fluid of such patients is able to induce a diminution of the specific absorption band. This observation indicates that the substances diffusing

SUMMARY

Incubation of buffered specimens of cerebrospinal fluid from patients who had undergone cerebral concussion with sections of cats' cords produced tigrolysis in the anterior horn cells, while similar treatment of the sections with buffered specimens of cerebrospinal fluid from normal persons or with buffered Ringer's solution failed to produce such an effect.

The findings point to the importance of enzymatic substances in the genesis of chromatolysis following cerebral concussion.

The nucleoproteins of the nerve cells go into solution at the hydrogen ion concentration prevailing in the normal central nervous system in vivo. When sections of spinal cords are incubated with various fluids, these fluids must be acidified for demonstration of Nissl bodies.

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from the central nervous system into the subarachnoid space after concussion are not only nucleases causing a breakdown of nucleic acids but also substances acting on their nitrogen-containing decomposition products (desaminases, according to J. B. Summer and G. F. Somers [Chemistry and Methods of Enzymes, New York, Academic Press, 1943, p. 103]).

EXPERIMENTAL MEDIONECROSIS OF THE AORTA

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DISSEMINATED medionecrosis of the aorta of man, a common cause of spontaneous rupture of the ascending aorta and of dissecting aneurysm, has been carefully studied by many workers.[1] The first systematic pathologic reports were made by Gsell [1a] and Erdheim.[1b] One of the best reports is that of Rottino and his co-workers.[1c,d] Erdheim gave this entity the name "medionecrosis cystica idiopathica" because of the necrosis present in the muscular and the elastic tissue and the mucoid-cystic degeneration of the media.

Experimentally, necrotic and degenerative changes of the media of the abdominal aorta and the peripheral arteries have been observed in rabbits by many investigators, following crushing of the vascular wall, sheating of the vascular wall in paraffin, dissecting off of the adventitia and applying of acids and thermocautery to the outside of the vascular wall.[2] One of the most careful and elaborate studies was

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* Fellow of the Dazian Foundation.

This investigation was aided by the A. D. Nast Fund for Cardiovascular Research.

From the Cardiovascular Department, Michael Reese Hospital. This department is supported in part by the Michael Reese Research Foundation.

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2. (*a*) Malkoff, G. M.: Beitr. z. path. Anat. u. z. allg. Path. **25**:431, 1899. (*b*) d'Anna, E.: Policlinico **4**:62, 1897. (*c*) Lange, F.: Virchows Arch. f. path. Anat. **248**:463, 1924. (*d*) Andriewitsch: Inaug. Dissert., St. Petersburg, 1901. (*e*) Fabris, A.: Virchows Arch. f. path. Anat. **165**:439, 1901. (*f*) Sumikawa, P.: Beitr. z. path. Anat. u. z. allg. Path. **34**:242, 1903. (*g*) Hildebrandt, F.: Experimentell erzeugte lokale Atherosklerose und ihre Beziehungen zur

that of Lange.[2e] Many agents, such as parenterally injected epinephrine hydrochloride, produced medionecrotic lesions in rabbits' arteries.[3]

In dogs, medionecrotic lesions could not be produced until recently, when Hueper and Ichniowski [4] reproduced such lesions in the aorta and the large arteries by injecting lethal and sublethal doses of histamine dihydrochloride. The lesions resembled closely those in the human

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aorta described by Gsell and Erdheim and those which follow burn shock.[5]

The formation of aneurysms has been observed in rabbits after injection of epinephrine hydrochloride.[6] There are also reports of aneurysm formation in cholesterol-fed rabbits.[7] Leary and Weiss[8] observed a dissecting aneurysm of the aorta arising in an atheromatous ulcer in a cholesterol-fed rabbit, which lived for three years. However, there are no reports of spontaneous rupture or dissecting aneurysm of the aorta due to necrotic changes of the media in dogs.

My associates and I became interested in the possibility of reproducing these necrotic lesions in dogs by interfering with the vascularization of the aorta. Since medionecrotic lesions of the aorta and the consequent spontaneous rupture and dissecting aneurysms usually are found in the ascending aorta, we concentrated our studies on this portion of the aorta.

METHOD

In 7 dogs the adventitia of the ascending aorta was coagulated by means of a specially devised simple cautery brought to a red glow over the flame of a Bunsen burner. Dogs weighing 20 to 30 pounds (9 to 13.5 Kg.) were used. During anesthesia (pentobarbital sodium, 25 mg. per kilogram) and artificial respiration, the chest was opened aseptically by a 4 to 7 cm. incision in the third or fourth right intercostal space beginning medially 2 cm. from the sternal margin. The pericardium was incised, the ascending aorta exposed and the fat pad carefully resected. An area of 2 sq. cm. of the adventitia was then coagulated in the region in which our previous studies had shown that the vasa arising from the right coronary artery anastomose most frequently with those from the left coronary artery and those from the brachiocephalic vessels of the arch. The thorax was then closed in layers with cotton thread, the pericardial sac being left open, and the pneumothorax was relieved. The dogs were permitted to recover from the anesthesia. They were killed after one, two or six weeks.

Two other dogs, previously made hypertensive by daily injections of desoxycorticosterone acetate while being permitted to drink 1 per cent sodium chloride solution ad libidum,[9] were used in this study. In both animals the blood pressure reached 225 mm. of mercury systolic and 125 mm. diastolic. One was used as a control; the other underwent cauterization of the aorta and was killed six weeks later.

The changes in the aorta in the first six to twelve hours after coagulation were studied in 4 dogs. In these animals, the chest was widely opened, and artificial respiration and anesthesia were maintained until they were killed.

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Autopsies were made on all the animals studied. The vasa of the aorta were injected; the aorta was then dissected, and roentgenograms were taken in the manner previously described. Microscopic sections of the aortas fixed in solution of formaldehyde U.S.P. were prepared and stained with hematoxylin and eosin, orcein, Masson and Van Gieson stains.

RESULTS

All the dogs survived the operation. Those permitted to come out of anesthesia behaved normally within a few hours after the operation with 1 exception. The exceptional animal refused food and water and was apathetic on the third post-operative day; it recovered in the next two days and behaved normally until killed fourteen days after the operation.

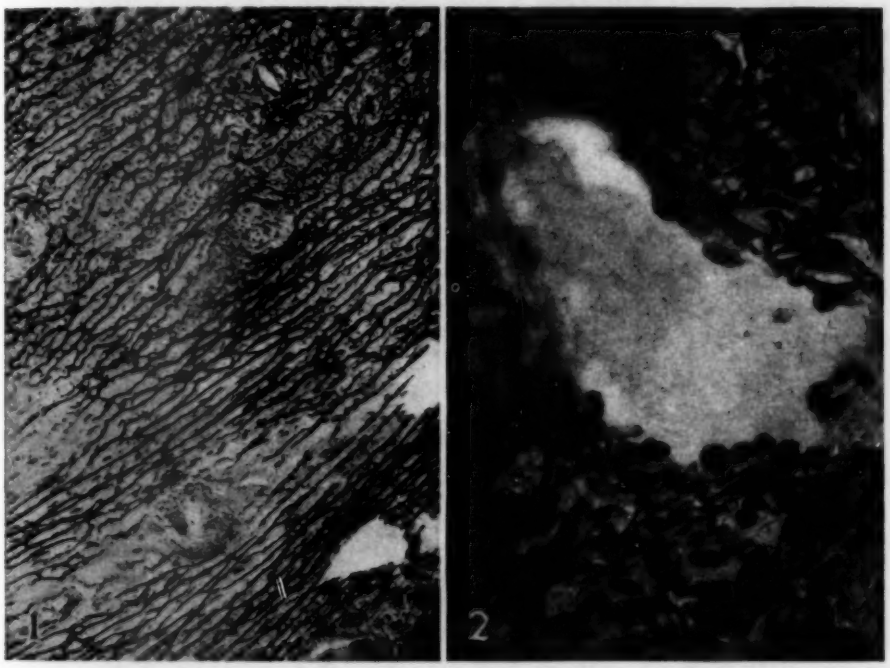

Fig. 1.—Outer and middle third of the aortic media showing diffuse necrosis. There are loss of nuclei, homogenization of the media and stretching and swelling of the elastic fibers. The disintegration of muscular tissue is accompanied by swelling, fusion, fragmentation and clumping of elastic fibers. There are areas of liquefaction with cyst formation, with or without mucoid material. Hematoxylin-eosin stain; × 80.0.

Fig. 2.—Middle third of the media showing cyst filled with mucoid material. Hematoxylin-eosin stain; × 400.0.

Aside from the adventitial coagulation, no gross alterations were seen in the aorta at the end of six to twelve hours. Microscopically, however, small hemorrhages were visible in the outer third of the media. The inner third of the media showed marked edema with separation of the elastic and muscle fibers (fig. 1). The intima showed no changes. In no instance could necrosis or cellular infiltration be seen in the media.

At the end of one to six weeks the pericardium and the right auricle were more or less adherent to the coagulated part of the aortic adventitia, the degree of adherence being more pronounced the longer the dogs survived.

One week after the operation the aorta on section showed yellowish brown areas in the media. These were noted not only beneath the coagulated adventitia but also beneath normal adventitia as far away from the coagulation as 1 to 2 cm. The intima showed no gross changes.

Two to six weeks postoperatively the gross changes were similar in location but the yellowish brown areas were partially replaced by grayish areas. The extent of the grayish areas increased the longer the dog survived. At the end of six weeks these grayish areas extended into the intima.

The dog which had been indisposed postoperatively showed at necropsy, at the end of two weeks, a bulging of the adherent area which was moderately soft to touch and extended the whole length of the ascending aorta. Careful examination revealed a rupture of the aorta, 0.5 mm. in length, 2 cm. above the aortic cusps, which had caused a dissection up to the arch. This dissecting aneurysm extended a short distance through the media and then between the media and the adventitia.

In the hypertensive animal (given desoxycorticosterone acetate) a large intimal plaque was found six weeks postoperatively. This plaque occupied the whole aortic circumference and measured approximately 4 sq. cm. The aorta in this region was inelastic and felt thinner. No yellowish brown areas were seen in this dog.

The microscopic observations were as follows: One week postoperatively the outer and middle thirds of the media were diffusely necrotic. This was evidenced by loss of nuclei, homogenization of the media and stretching and swelling of the elastic fibers. The disintegration of muscular tissue was accompanied by swelling, fusion, fragmentation and clumping of elastic fibers (fig. 1). Areas of liquefaction with cyst formation, with or without mucoid material, were observed (figs. 2 and 3). These necrotic areas appeared not only beneath the coagulated adventitia but also beneath normal adventitia (fig. 4). They were noted also in the middle part of the media, surrounded by normal media (figs. 4 and 5). On the other hand, normal media was noted below the coagulated adventitia. With the latter one could observe blood vessels filled with contrast dye in the media. These blood vessels must have derived their supply from the collateral circulation via the extensive anastomotic channels,[10] since the vessels of the adjacent adventitia had been destroyed by the coagulation. In every case of diffuse necrosis the blood vessels in the necrotic areas were not injectable. A few hemorrhages could be seen filling spaces which had undergone liquefaction, and these were located near the line of demarcation between necrotic and normal media. The inner third of the media showed focal necrosis with a predominance of cystic changes, but revealed no diffuse homogenization. Fragmentation, granulation and clumping of elastic fibers in these areas were predominant. The focal lesions showed a distribution similar to that observed in the middle and outer media. In many instances young collagenous fibers started to replace the necrotic tissue and the albuminoid material of the cysts. No instance of cellular infiltration of the media was observed. Except for 1 dog with two areas of slight intimal proliferation, no pathologic alteration of the intima was noted (fig. 5).

In the second week the medial changes were similar to those noted in the first week with some additions. However, no hemorrhages were observed. The additional changes consisted of (*a*) much more marked breakdown of elastic

10. Schlichter, J. G.: Am. Heart J., to be published.

fibers, (*b*) replacement of cystic and necrotic areas by young fibrous tissue, (*c*) increase of the cystic areas in the inner third of the media, (*d*) appearance of fibrous tissue growing into the media from the adventitia and (*e*) more marked intimal proliferation above the necrotic areas.

One of the dogs which, as previously mentioned, showed rupture of the aortic wall had necrosis of the whole aortic wall and homogenization in this area. The intima around the rupture showed thickening and marked proliferation (fig. 7). The rupture extended from the intima to the media and had dissected between the latter and the adventitia (fig. 8). Fibrous tissue was seen growing into the rupture from the adventitia, and organization of thrombotic material was noted.

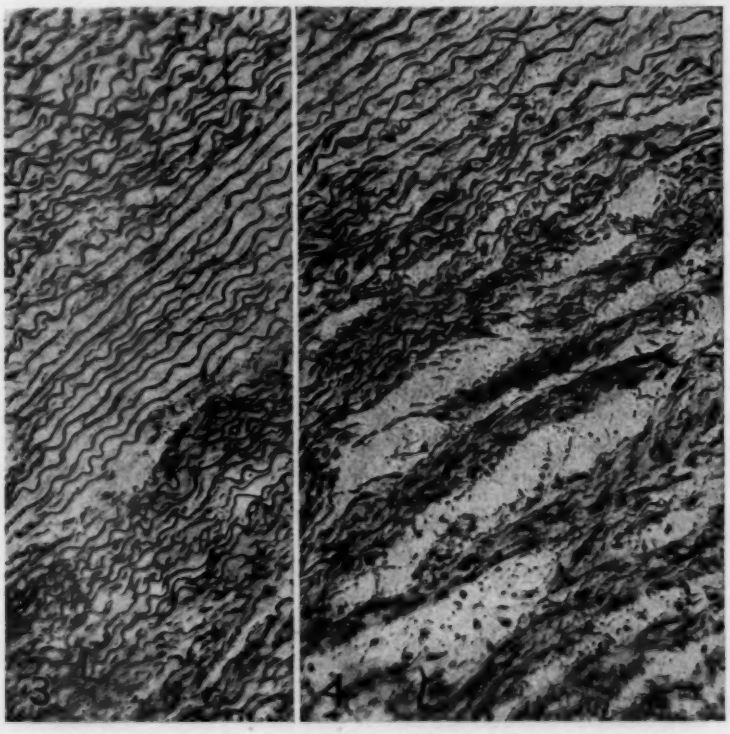

Fig. 3.—Middle third of the media showing necrosis of the muscular tissue with stretching and swelling of elastic fibers. The necrotic area is surrounded by normal media and, beneath, normal adventitia. Hematoxylin-eosin stain; × 132.

Fig. 4.—Necrosis of the outer third of the media is present, and cystic and necrotic areas are being replaced by fibrous tissue. Hematoxylin-eosin stain; × 144.

Hyalinization of the walls of some vasa with luminal thrombosis was observed in the adventitia at a spot next to the coagulation.

In the fourth to sixth week postoperatively, the replacement of necrotic tissue by collagenous and fibrous tissue was pronounced, and intimal proliferation was marked above the necrotic areas. The necrotic areas were replaced more and more by fibrous tissue, which formed an irregular meshwork in the middle and inner thirds of the media (fig. 9). The cystic areas were replaced or filled by

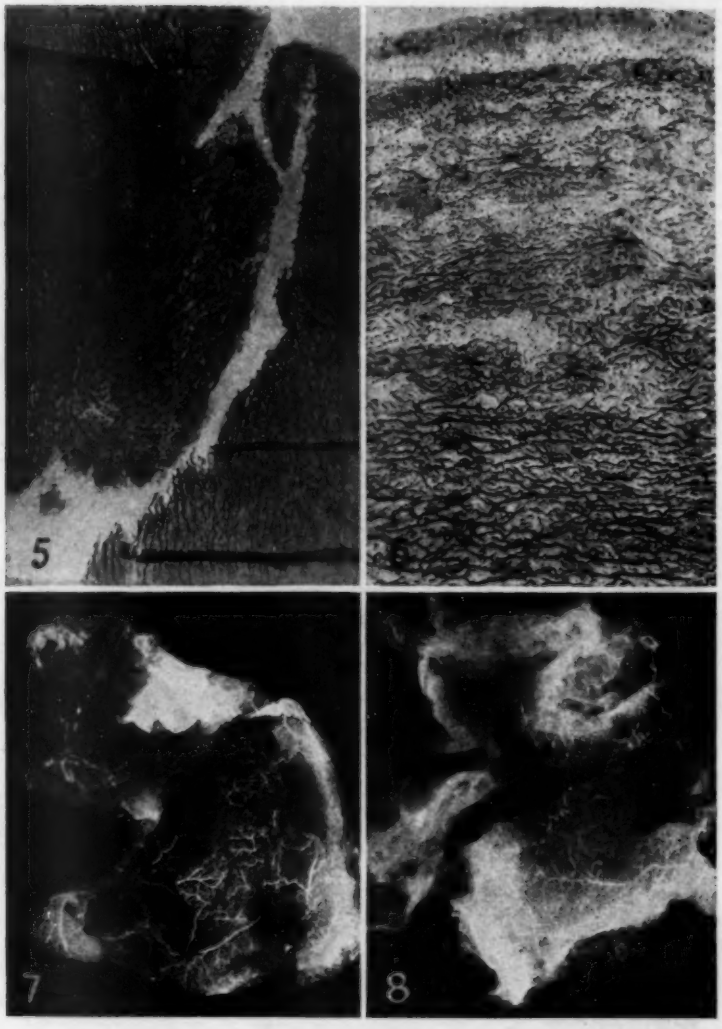

Fig. 5.—Necrosis of the whole aortic wall with homogenization of the media. There is a rupture of the aortic wall. The intima around the rupture shows thickening and proliferation. Fibrous tissue is growing into the rupture from the adventitia, and organizing thrombotic material can be noted. Hematoxylin-eosin stain; × 16.

Fig. 6.—Extensive proliferation of the intima in the hypertensive dog. Hematoxylin-eosin stain; × 61.6.

Fig. 7.—Marked increase and irregularity of the vascularity of the coagulated aorta of the hypertensive dog six weeks after coagulation. Note the presence of a vessel arising directly from the intima, proved by histologic examination.

Fig. 8.—The injected ruptured aorta shows no vessels in the coagulated area, and there are no anastomoses between the right and the left coronary artery. The arcuate branches of the coronary arteries are also severed.

Note.—This photograph, in the course of reproduction, was turned. What now is the left side should be the top.

connective tissue (fig. 10). Hyalinization of small areas of the inner third of the media and of the intima was observed, and these areas protruded into the lumen of the aorta. The fibrous tissue growing into the adventitia and the outer third of the media was richly vascularized, the vascularity being much more pronounced than that observed in normal dogs. Nevertheless, no filling of medial vessels could be seen in these areas.

The changes in the hypertensive dog were the same except that the proliferation of the intima (fig. 11) was much more marked and a vessel opening directly from the lumen of the aorta was observed in the area above the medial alterations. Incidentlly, the control, hypertensive dog revealed no abnormal changes.

Roentgenograms of the aorta revealed that anastomoses between vasa arising from the left and from the right coronary artery and vasa from the vessels of the aortic arch were still present (fig. 12). Because of the rich anastomoses, the coagulation had not completely interrupted the blood supply. Six weeks after coagulation of the adventitia, the vascularity of the aorta appeared as a dense anastomotic network in the coagulated area. The intimal vessel direct from the lumen of the aorta in the hypertensive dog has already been mentioned (fig. 13).

In the dog with aortic rupture, unlike the others, complete absence of vascularity was found in the area which ruptured. No anastomoses or collaterals appeared to exist in this animal between the vasa arising from the left and the right coronary artery or between them and the arcuate branches of the coronary arteries, which could supply the ruptured area (fig. 14).

COMMENT

These studies have revealed some of the factors involved in the changes in the structure of the aortic wall when its vascularity is experimentally interfered with. The presence of an extensive collateral circulation minimizes the degenerative process following interference with the adventitial vascularization or, at least, retards it. Because of the slowing up of the degenerative processes, mucoid degeneration and cysts form in the outer and middle thirds of the media. Necrosis of the whole wall develops only when the circulation of the areas is completely severed and the supply through stomas is minimal. Such extensive necrosis can lead to rupture, and the rupture can dissect slowly to form a dissecting aneurysm. This actually occurred in a dog observed in this study. On the basis of the clinical findings in this dog it would appear that the rupture developed on the third postoperative day. The tendency to heal was evidenced by the presence of fibrous tissue growing into the rupture and by the organization of thrombotic material. Cases of healed rupture of the human aorta have been reported ([1m]).

No instance of cellular infiltration of the media was found. The necrotic and the cystic areas were replaced by collagenous and fibrous tissue, and new vascularization of the adventitia appeared. This process seemed to be complete by the fourth to sixth postoperative week. The revascularization is more abundant than that in normal dogs, although it is irregular in its extent. One could not demonstrate any new medial network in these areas, at least of vessels injectable with the dye. Smaller

vessels, below 10 microns, were seen microscopically. In 1 case, a vessel opening directly from the lumen of the aorta was seen filled with dye. It is not possible to state whether or not this vessel was newly developed or had existed before operation. In several instances it was apparent that the presence of large vessels arising from the intima, or of collaterals and anastomoses from uncoagulated adventitia, tended to prevent the development of medial necrosis. On the other hand, necrosis developed above areas of normal adventitia if these areas belonged to the supply of coagulated vessels and had insufficient collaterals.

Some of the animals in which the adventitial coagulation was superficial in areas showed hyalinization with thrombosis of vasa vasorum.

The hypertensive dog showed more extensive proliferation of the intima than the other dogs and a few areas of hyalinization in the intima and the outer third of the media. The wall below the coagulated adventitia could be considered a small true aneurysm; it was thinner, inelastic and had marked fibrous replacement. Desoxycorticosterone acetate was used in this dog, and it has been reported to cause medionecrosis. However, the control dog to which this drug was given, but which had no adventitial necrosis, showed nothing unusual in its aorta. Hypertension is important in the after-effects of medionecrosis. It weakens the aortic wall involved by this lesion and leads to a greater tendency toward aneurysmal dilatation. By putting a greater strain on the aorta, it leads to a greater chance of rupture of the previously necrotic wall. Even in normotensive subjects, however, there is enough rapid and marked fluctuation in blood pressure under conditions of emotional or physical strain or with coughing to lead to rupture of a necrotic aortic wall (Schlichter [11]).

Heuper and Ichniowski [4] reproduced degenerative and cystic changes in the dog's aorta by subjecting the animals to lethal or sublethal histamine shock. Dogs treated with repeated intravenous injections of excessive doses of epinephrine hydrochloride did not show arterial lesions. Otto [3m] found that administration of epinephrine extending for long periods led to fibrous thickening of the intima and some medial degeneration. The rabbit is a much more suitable animal for the reproduction of medionecrotic changes resembling those in man. Many endogenous and exogenous agents, epinephrine being the most commonly employed substance, have produced medionecrotic lesions in the aorta and other arteries of the rabbit. Hueper,[12] in his recent review, stated that it is generally conceded that acute epinephrine-

11. Schlichter, J. G.: Beitrag zu den Aneurysmen und Rupturen des Herzen, Thesis, Lausanne, University of Lausanne, 1940.

12. Hueper, W. C.: Arch. Path. **38**:162, 245 and 350, 1944; **39**:51, 117 and 187, 1945.

induced arterionecrosis resembles somewhat arteriocalcinosis of the Mönckeberg type. In man, medionecrosis of the aorta was observed with many diseases, including coronary disease, infectious disease, hypertension, hypotension and endocrine disorders. Ziegler [3p] and Pearce and Baldauf [3g] concluded that medionecrosis is the result of ischemia following circulatory disturbances of the vasa vasorum. This mechanism was also emphasized by Erdheim [1b] and by Schlichter.[11] The present studies have clearly revealed that this mechanism is operative. In comparing the medionecrotic lesions reproduced in different species by different methods with the lesions in man one concludes that the vascularization of the aorta determines the site, the extent and the severity of these lesions.

The difference in susceptibility to ischemia of the aortic wall in different species is due to the difference in the richness of the vascularization of the aorta. I have found that the rabbit has the poorest vascularization, the dog the best, while that of man is in between. In rabbits vessels over 10 microns are scarce in the aorta. In the media no vessels of this size could be demonstrated. In man the adventitial and medial networks are much less abundant than in the dog. In fact, no extensive medial vasculature of vessels over 10 microns could be demonstrated in the human aorta, nor could such-sized vessels be seen arising directly from the lumen of the aorta. The excellence of the aortic vascularization of the dog may explain why medionecrosis, as well as arteriosclerosis, is so difficult to produce in this species. On the other hand, the poor vascularization of the rabbit's aorta may account for the ease of reproducing medionecrosis and its sequelae (and arteriosclerosis) in this species.

The healing of the medionecrotic lesions and ruptures depends on the adequacy of the collateral circulation. This is sufficiently variable, and so is the primary occlusion, to account for the variability in healing encountered. In the present studies of the vascularity of the dog's aorta I was impressed with the variability of the collateral circulation observed in different animals.

The individual variability of vascularization of the descending aorta is less striking than that of the ascending aorta. This may be an important factor in explaining why necrosis followed by rupture and dissecting aneurysm is more common in the ascending than in the descending aorta. However, medionecrosis of the type found in the ascending aorta does occur in the descending aorta and the arch, as Rottino [1c] has shown. On routine postmortem examination a large number of such lesions were encountered by him, distributed throughout the aorta. However, it is rarer to find rupture and dissecting aneurysm arising in the arch or the descending aorta, even though these regions have a poorer vascularity than the ascending aorta. It may be that the greater frequency of

rupture and dissection of medionecrosis in the ascending aorta is due, aside from the individual variability of the vascularization of the ascending aorta, to (*a*) the greater mobility of this region within the pericardial sac, (*b*) the possibility of impact of the stream of blood leaving the heart and impinging on its walls and (*c*) the fact that, unlike the descending aorta and the arch, the ascending aorta is not surrounded by connective tissue from the neighboring structures, which has a strengthening action on the walls. Another factor of importance may be the greater frequency of stomas in the intima beyond the ascending aorta. Therefore, compared with the adventitial supply, the relative supply directly from the lumen is even more abundant. This insures a better compensatory system to make up for interruptions in blood supply from the adventitia and prevents total necrosis of the aortic wall.

The role of these peculiarities in the vascularity of different portions of the aorta may also be related to the marked preponderance of atheromatous lesions in the descending aorta.

SUMMARY

The adventitia of the ascending aorta was coagulated in dogs, and the changes in the aortic wall were observed after from six hours to six weeks.

Edema appeared in the inner third of the media and small hemorrhages in its outer third within twelve hours after coagulation.

In the outer and middle thirds of the media, in which the blood supply had been interfered with, diffuse necrosis was observed in the majority of the dogs. In the inner third of the media, which is supplied to a large extent from the lumen of the aorta, only focal necrosis and cyst formation appeared. The process of necrosis was a slow one, involving the muscular constituents before the elastic fibers. It was followed in turn by liquefaction, cyst formation and collagenous fiber replacement. The extent and the speed of necrosis depended on the efficacy of the collateral circulation. Usually the process ended in the third week. There was new formation of blood vessels in the adventitia, and collateral channels developed. Adhesion of the split pericardium also helped in the revascularization.

Proliferation of the intima appeared in the second week, was most extensive in the sixth week and paralleled the medial repair process.

In 1 dog a spontaneous rupture and a dissecting aneurysm of the ascending aorta were produced. In another dog, a hypertensive one, a true small aneurysm appeared.

The incidence of medionecrosis, spontaneous rupture and dissecting aneurysm of the ascending aorta seen in dogs was compared with that seen in man and other species, and with the incidence of such lesions of other parts of the aorta.

COURSE OF WOUND HEALING IN THE SKIN OF MICE UNDER THE INFLUENCE OF CARCINOGENS

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ST. LOUIS

PROLONGED application of 3,4-benzpyrene or of 20-methylcholanthrene previous to the making of wounds in the skins of young mice resulted in retardation of the closure of the wounds.[1] The delay was due to an inhibition of the migration of the epidermal cells which normally advance into the defects. This delay was the more conspicuous as cell proliferation was markedly increased at the margin of the excision and the mitotic cycle of this epithelium failed to return to normal ten days after the wounds had been made, as that of epithelium of untreated skin did.

Among the questions arising from these observations were the following: (1) Is the cell migration only temporarily suspended, or is it permanently inhibited? (2) Provided complete epithelization does finally occur, would the mitotic counts remain high because of the stimulative effect of the carcinogenic hydrocarbons, or would they drop to normal levels? The answer to the last question seemed of interest also in regard to the problem as to whether a surgical wound may influence the localization of tumors in epidermis treated with such substances. We have therefore undertaken a study of the late stages of wound healing in the skins of young mice treated with carcinogenic hydrocarbons.

MATERIAL AND METHODS

Sixty-four mice of the inbred strain RFI, 8 weeks old at the beginning of the experiments and kept on a standard diet of Purina dog chow [2] and water,

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This investigation was aided by the David May–Florence G. May Fund.

From the Snodgras Laboratory of Pathology, City Hospital, and the Pathological Laboratory of the Jewish Hospital.

1. Silberberg, M., and Silberberg, R.: Am. J. Path. **20**:809, 1944; Arch. Path. **39**:257, 1945.

2. The Ralston Purina Company supplies the following list of ingredients of the dog chow: meat meal, dried skim milk, riboflavine, carotene, cod liver oil,

(Footnote continued on next page)

were used, and litter mate animals of both sexes were as evenly divided among the experimental groups as possible. The two main groups included (1) 32 mice painted with a benzene solution of 20-methylcholanthrene (concentration 0.3 per cent) and (2) 32 mice treated with a benzene solution of 3,4-benzpyrene (concentration 0.3 per cent). These two solutions of carcinogenic substances were applied three times weekly, each application being made with a single stroke of a camel's hair brush, no. 6, on a carefully clipped area of skin of the back. In each group subgroups of 8 animals each were thus treated for two weeks, one month, two months and three months, respectively. At the end of the period of treatment a circular piece of epidermis with some underlying tissue, measuring 4 mm. in diameter, was excised from each animal with a pair of curved scissors. Healing of the wounds was allowed to take place, and ten, twenty, thirty or forty-five days after the excisions the animals were killed in pairs between 10 and 11 a. m. The skin containing the scar and adjacent tissue was fixed in solution of formaldehyde U. S. P. diluted 1:10, and serial sections were prepared for histologic study. The further details are the same as those given in previous papers.

HISTOLOGIC EXAMINATION

CONTROLS

As previously shown in untreated control mice, the skin consists of two layers of epidermal cells. In 10,000 basal cells 12 mitoses (a mean) were counted, the ratio between basal and spinous cells being 2:1. The wounds, 4 mm. in diameter, made in the skins of these untreated mice were epithelized ten days after excision.

MICE PAINTED WITH 20-METHYLCHOLANTHRENE

Wound Healing After Two Weeks of Application.—Marginal Epithelium: Ten days after excision (table 1) the epidermis consisted of four or five rows of epithelial cells, which were larger than those of the untreated controls. Whereas in the latter the number of mitoses had dropped to normal, in the painted animals the mean mitotic count was two and one-fourth times higher. After twenty or thirty days there were still three to five cell rows and two and one-fourth or two and three-fourths as many mitoses as ordinarily. Forty-five days after operation the mean mitotic count had fallen to one and one-fourth times the normal; this coincided with a decrease in the number of rows of epithelial cells to three or two. The details are given in table 1.

New Epithelium: Ten or twenty days after the making of the wounds the defects were closed and covered by from five to eight rows of large epithelial cells. The number of mitoses was normal or slightly above normal with the exception of animal 350, in which the epithelium was exceedingly thick (seven

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brewers' dried yeast, wheat germ, wheat cereal, corn grits, corn cereal, dried beet pulp, molasses, steamed bone meal and iodized salt. The chemical analysis is:

	Crude, %	Digestible, %
Protein	23.0	19.0
Fat	5.0	4.7
Fiber	4.0	
Ash	7.0	
Nitrogen-free extract	54.0	48.0
Moisture	7.0	

or eight rows of cells) and the number of mitoses four times the normal. After thirty or forty-five days the epidermis was composed of two or three rows of cells, thus approaching the usual condition, while the mean mitotic counts had dropped to one and a half or one-third the usual values. Even then, however, the scar could be recognized in the section by the presence of more fibrous tissue in the subcutis and by the absence of appendages.

Wound Healing After One Month of Application.—Marginal Epithelium: Ten days after excision the epidermis was thickened (four to eight rows of cells), and in the enlarged cells the mean count of mitoses was five and a half times the normal. The epithelium was covered by a layer of keratin. From twenty days after the operation on, the epithelium became much thinner (two to four rows of cells), and the number of mitoses likewise showed a return to normal.

New Epithelium: In animal 335 the wound was about to close, whereas in mouse 336 the defect was completely epithelized. In the former the epidermal layer was thicker (nine rows of cells) and more mitoses (nine times the normal) were found than in the latter, which showed six or seven rows of cells and five times the normal number of mitoses. After twenty or thirty days there were only four or five rows of epithelial cells and the means of mitotic counts were lower than before (two and one-fourth and one and three-fourths the normal, respectively). After forty-five days the epidermis consisted of two or three rows of small epithelial cells, with a mean mitotic count of half the usual values only.

Wound Healing After Two Months of Application.—Marginal Epithelium: Ten or twenty days after the making of the wound there were four or six rows of epithelial cells covered by a thick layer of keratin, and the mean mitotic counts were five and a half or six and one-fourth times as high as ordinary. After thirty or forty-five days, however, the keratinization was less accentuated; the epidermis was composed of only three or four rows of epithelial cells, and the numbers of mitoses had declined to a mean of three, or one and three-fourths times the normal.

New Epithelium: After ten days the defects were still open in both animals. The epithelium migrating into the wound contained six to eight cell rows, and the mean mitotic count was three and three-fourths times the normal. Twelve to fourteen days after excision a marked narrowing of the wounds had been noted in the living animals; after sixteen days the crusts covering the defects were sloughed off and scars became visible. Histologically, after twenty days the number of epithelial cell rows had decreased to four or five, and the numbers of mitoses had dropped to a mean of one and three-fourths times the normal. Subsequently there was a further thinning of the epithelium with a further decrease in the numbers of mitoses, the mean of which was three-fourths the normal forty-five days after operation. In 3 of the animals observed for thirty or forty-five days after excision papillomas had developed at some distance from the scars. The healing of the wounds was apparently not influenced by the presence of these tumors.

Wound Healing After Three Months of Application.—Marginal Epithelium: Ten days after the wounds had been made the epithelium consisted of six to ten rows of epithelial cells, above which there was a thick layer of keratin. The mean mitotic count of the basal cells was as high as ten times the normal. At twenty days and later the number of the epithelial cell rows decreased somewhat, to from five to eight layers; there was less keratinization, and the numbers of mitoses dropped likewise. The mean mitotic counts were from eight and a half

TABLE 1.—*Methylcholanthrene-Treated Animals*

Duration of Painting	Duration of Healing	Animal	Rows of Cells in Old Epith.	Rows of Cells in New Epith.	Mean Number of Mitoses with Their Maximum and Minimum Deviations in Multiples of the Normal: Old Epith.	Mean Number of Mitoses with Their Maximum and Minimum Deviations in Multiples of the Normal: New Epith.	Type of Tumor
2 weeks	10 days	348	4	6	2½ max. 3½ min. 1½	1 max. 1 min. 1	
		349	5	6	2 max. 2 min. 2	1½ max. 2 min. 1	
					Mean 2¼	Mean 1¼	
	20 days	350	4-5	7-8	2½ max. 3 min. 2	4 max. 4½ min. 3	
		351	3-4	5-6	2 max. 3 min. 2	1½ max. 2 min. 1	
					Mean 2¼	Mean 2¾	
	30 days	352	3	3	1½ max. 2 min. 1	½ max. 1 min. ½	
		353	4	3	4 max. 5 min. 3	2½ max. 3 min. 2	
					Mean 3¾	Mean 1½	
	45 days	354	2-3	2-3	1½ max. 2 min. 1	½ max. ½ min. 0	
		355	2-3	2	1 max. 1 min. 1	½ max. ½ min. 0	
					Mean 1¼	Mean ½	
1 month	10 days	335	8	9	6 max. 9 min. 5	9 max. 10 min. 7	
		336	4-5	6-7	5 max. 6 min. 3½	5 max. 6 min. 4½	
					Mean 5½	Mean 7	
	20 days	337	2-3	4-7	1 max. 1 min. 1	3 max. 4½ min. 2½	
		362	3	4	1 max. 1 min. 1	1½ max. 2 min. 1	
					Mean 1	Mean 2¼	
	30 days	356	3	4	1 max. 1 min. 1	2 max. 2½ min. 1	
		357	4	5	2 max. 3 min. 1	1½ max. 2½ min. 1	
					Mean 1½	Mean 1¾	
	45 days	358	4	3	1 max. 1 min. 1	½ max. ½ min. ½	
		359	2	2-3	1 max. 1½ min. 1	½ max. ½ min. ½	
					Mean 1	Mean ½	
2 months	10 days	363	4	6	4½ max. 5 min. 3	3 max. 4 min. 2½	
		364	6	8	6½ max. 7½ min. 5½	4½ max. 6 min. 4	
					Mean 5½	Mean 5¾	
	20 days	365	6	4-5	6 max. 7 min. 5	1½ max. 2 min. 1	
		366	5	4	6½ max. 8 min. 6	3 max. 2½ min. 1½	
					Mean 6¼	Mean 1¾	
	30 days	367	3	6	3 max. 4 min. 2½	2 max. 2½ min. 1	Papillomas
		368	3-4	2-3	3 max. 4 min. 2	2 max. 2½ min. 1½	
					Mean 3	Mean 2	
	45 days	369	3-4	3	1½ max. 2 min. 1	½ max. 1 min. ½	Papilloma
		370	4	3-4	2 max. 2½ min. 1½	1 max. 1½ min. ½	Papilloma
					Mean 1¾	Mean ¾	

Duration of Painting	Duration of Healing	Animal	Rows of Cells in Old Epith.	Rows of Cells in New Epith.	Mean Number of Mitoses with Their Maximum and Minimum Deviations in Multiples of the Normal: Old Epith.	Mean Number of Mitoses with Their Maximum and Minimum Deviations in Multiples of the Normal: New Epith.	Type of Tumor
3 months	10 days	338	6	10	6 max. 8 min. 4½	7½ max. 8½ min. 6½	Papillomas
		339	10	12	14 max. 16 min. 12	17 max. 20 min. 14	Papilloma
					Mean 10	Mean 12¼	
	20 days	340	5	7	9½ max. 15 min. 6	4 max. 4½ min. 3½	Papillomas
		341	7	5	7½ max. 8 min. 7	4 max. 5 min. 3	Papillomas
					Mean 8½	Mean 4	
	30 days	342	7	9	5 max. 6 min. 4	3 max. 4 min. 3½	Papillomas
		343	6	8-9	6½ max. 8 min. 4½	2½ max. 3 min. 2	Papillomas
					Mean 5¾	Mean 2¾	
	45 days	344	8	10	4 max. 5½ min. 4	2½ max. 3 min. 1½	Papillomas
		345	6	4	4 max. 5 min. 2½	5 max. 6 min. 4	Papillomas
					Mean 4	Mean 3¾	

to four times higher than usual and showed wide variations. This was apparently due to the presence of tumors, which had developed near the wounds as well as at some distance. The closer the neoplasms to the regenerating epithelium, the more numerous were the mitoses in the marginal epithelium; the farther away the new growths were, the fewer were the mitoses at the edges of the excisions. No tumor was noted in the wounds or in the scars themselves.

New Epithelium: The wounds were still open after ten days. However, they narrowed markedly at the end of the second week, and they were healed after twenty days. Histologically, ten days after operation the epithelium contained from ten to twelve rows of cells, and the mean mitotic count was twelve and one-fourth times higher than usual. Subsequently, the epithelial layer in the scars decreased in size, but close to the tumors the epithelium remained markedly thickened. The numbers of mitoses showed likewise a decline, and after forty-five days a mean of three and three-fourths times the normal was found. Again the mitoses were the more numerous the closer the scars were to the tumors. In some mice multiple papilloma had developed, which accounted for wide variations in the numbers of mitoses, particularly if the new growth approached the regenerated area, but in no case did a tumor originate in the area of excision.

MICE PAINTED WITH 3,4-BENZPYRENE

Wound Healing After Two Weeks' Application.—Marginal Epithelium: Ten days after operation the epidermis showed a surface layer of keratin and contained three or four layers of epithelial cells (table 2). The mean mitotic count of the basal cells was four and three-fourths times the normal. During the late stages the epithelium decreased slightly in thickness (two to four rows of cells) and the mean of the mitotic counts ranged between two and one-half and one and three-fourths times the usual figures. The details are presented in table 2.

New Epithelium: Ten days after excision the wounds were epithelized and covered by four to eight rows of large epithelial cells. The mean mitotic count

TABLE 2.—*Benzpyrene-Treated Animals*

Duration of Painting	Duration of Healing	Animal	Rows of Cells in Old Epith.	Rows of Cells in New Epith.	Mean Number of Mitoses with Their Maximum and Minimum Deviations in Multiples of the Normal: Old Epith.	Mean Number of Mitoses with Their Maximum and Minimum Deviations in Multiples of the Normal: New Epith.	Type of Tumor
2 weeks	10 days	373	4	7-8	5½ max. 6½ min. 4½	10 max. 12 min. 8	
		374	3	4-5	4 max. 4½ min. 3½	3½ max. 4½ min. 2½	
					Mean 4¼	Mean 6¾	
	20 days	375	3	4-5	3 max. 4 min. 2½	1½ max. 2 min. 1	
		376	4	4	2 max. 3 min. 2	1 max. 1½ min. ½	
					Mean 2½	Mean 1¼	
	30 days	377	4	3	1½ max. 2 min. 1	½ max. ½ min. 0	
		378	3-4	3	2 max. 2 min. 3	½ max. ½ min. ½	
					Mean 1¾	Mean ¼	
	45 days	379	3	4	3½ max. 4½ min. 2½	½ max. ½ min. 0	
		380	2-3	3	1 max. 1 min. 1	½ max. ½ min. 0	
					Mean 2¼	Mean ½	
1 month	10 days	383	4	5-6	3 max. 4 min. 2½	6 max. 7½ min. 5	
		384	5-6	8	6 max. 7 min. 5	11 max. 13 min. 10	
					Mean 4½	Mean 8½	
	20 days	385	4	4-5	5½ max. 6 min. 4	2½ max 3 min. 2	
		386	3-4	4-5	2½ max. 2½ min. 2	2½ max. 3 min. 2	
					Mean 4	Mean 2½	
	30 days	387	3-4	3	1½ max. 2 min. 1	½ max. 1 min. ½	
		388	3	2	1 max. 1½ min. 1	½ max. 1 min. ½	
					Mean 1¼	Mean ½	
	45 days	389	4	3-4	2 max. 2½ min. 2	½ max. ½ min. 0	
		390	4	3	1 max. 1 min. ½	½ max. ½ min. 0	
					Mean 1½	Mean ½	
2 months	10 days	392b	6	7-8	7½ max. 9 min. 6	11 max. 12 min. 10	Carcinoma
		393	6-7	9-10	7 max. 8 min. 6	11 max. 12½ min. 10	
					Mean 7¼	Mean 11	
	20 days	394	4	3	4½ max. 6 min. 4	1½ max. 2 min. 1	
		395	4-5	4	8 max. 9 min. 7	2½ max. 3 min. 2	
					Mean 6¾	Mean 2	
	30 days	396	6-7	4	11 max. 12 min. 10	11 max. 12 min. 10	Carcinoma
		397	4	9-10	10 max. 11 min. 9	15 max. 17 min. 14	Carcinoma and papilloma
					Mean 10½	Mean 13	
	45 days	398	6	5-6	2 max. 2 min. 1½	1 max. 2 min. ½	Carcinoma
		399	4-5	3-4	3 max. 4½ min. 2½	2 max. 2½ min. 1½	Carcinoma
					Mean 2½	Mean 1½	

Duration of Painting	Duration of Healing	Animal	Rows of Cells in Old Epith.	Rows of Cells in New Epith.	Mean Number of Mitoses with Their Maximum and Minimum Deviations in Multiples of the Normal — Old Epith.	Mean Number of Mitoses with Their Maximum and Minimum Deviations in Multiples of the Normal — New Epith.	Type of Tumor
3 months	10 days	402	5-6	7-8	10 max. 11 min. 10	14 max. 15 min. 13	Papilloecarcinoma
		403	7	5	8 max. 9½ min. 7½	12 max. 13 min. 11½	Carcinoma
					Mean 9	Mean 13	
	20 days	404	6	4-5	7½ max. 9 min. 6	10 max. 11 min. 9½	Carcinoma
		405	6	8	8 max. 10 min. 7	9½ max. 11 min. 7½	Carcinoma
					Mean 7¾	Mean 9¾	
	30 days	406	5	6-7	12½ max. 14 min. 11	14 max. 15 min. 13	Carcinoma and papillomas
		407	6-7	8	10 max. 10½ min. 10	10 max. 11 min. 9	Carcinoma and papilloma
					Mean 11¾	Mean 12	
	45 days	408	7	8-9	14 max. 15 min. 12	14 max. 15 min. 13	Carcinoma and papillomas
		409	6	6	12 max. 14 min. 10	12 max. 14 min. 10	Carcinoma and papillomas
					Mean 13	Mean 13	

was six and three-fourths times as high as ordinarily. After twenty days there were four or five rows of cells and a decline in the number of mitoses to one and one-fourth the normal. After thirty or forty-five days the scar showed three or four rows of small epithelial cells and a mitotic count of one-half or one-fourth the normal.

Wound Healing After One Month of Application.—Marginal Epithelium: Ten days after operation the epidermis contained four to six rows of epithelial cells, covered by a layer of keratin. Even after forty-five days of observation the epidermis remained thickened (three or four rows of cells). After twenty days the mean number of mitoses was still four times the normal. After thirty or forty-five days there was a steep drop in the mitotic counts to about normal.

New Epithelium: After ten days the defects were not yet completely covered with epithelium. The epithelial tongues advancing from the margins of the wound were composed of from five to eight rows of cells and the mean number of mitoses therein was eight and a half times the normal. Grossly, the defects were healed between fourteen and sixteen days after excision. After twenty days, coincident with a decline in the number of rows of epithelial cells to four or five, two and a half times (mean) as many mitoses were found as ordinarily. At later stages the epithelium became still thinner (three or four rows of cells), and the mean mitotic count dropped to below normal.

Wound Healing After Two Months of Application.—Marginal Epithelium: Carcinoma was present in 5 of the 8 mice. In 2 of the mice it was in such close approximation to the scar that the effect of healing on the mitotic count could not be determined. In the other 3 mice it was sufficiently distant from the line of excision to allow a study of the margin of the wound. Observations made in these 3 mice and on the remaining 3 mice, in which no tumors were present, provided the basis for the following description. Ten days after operation the epidermis was covered by a thick layer of keratin and consisted of six or seven

rows of epithelial cells with a mean mitotic count of seven and one-fourth times the normal. After twenty days the number of cell rows had decreased to four or five and that of the mitoses to a mean of six and one-fourth times the usual values. After forty-five days the mean mitotic count was down to two and one-half times the normal.

New Epithelium: Ten days subsequent to excision the defects were not yet epithelized. The epithelial tongues were thick and contained from seven to ten rows of cells showing a mean mitotic count of eleven times the normal. Subsequently, the wounds closed, but the numbers of mitoses showed wide variations. After twenty days and later the mean number of mitoses was slightly higher than ordinarily, the individual values ranging between normal and two and a half times the normal.

Wound Healing After Three Months' Application.—Marginal Epithelium: Only in the animals observed for ten days were the tumors sufficiently distant from the wounds so that cell counts could be made. All animals had carcinoma in the painted skin. Some had in addition multiple papilloma. Ten days after operation the epidermis consisted of five to seven layers of epithelial cells and a thick layer of keratin. The mean mitotic count was nine times as high as usual. Subsequently the number of mitoses remained high or even increased, particularly near the neoplasms.

New Epithelium: The wounds were not epithelized ten days after operation. The epithelial tongues consisted of five to eight rows of cells, the mean mitotic count being thirteen times the normal. Since at later stages tumors had grown over the scars, the high mitotic counts obtained cannot be considered as representing the true figures for mitoses in the scarred epithelium.

COMMENT

Wounds 4 mm. in diameter made in untreated skin of young mice were closed ten days after excision. As reported previously,[1] when methylcholanthrene or benzpyrene was applied to the epidermis for two weeks or for one month previous to the making of the defect, this treatment caused a slight acceleration of healing comparable to that seen after painting with the solvent benzene alone. If the carcinogens were applied to the epidermis as long as two or three months previous to the operation, healing of the excised area was completed after about fifteen days, that is, five days later than in nontreated epidermis. Carcinoma was present in every animal to the skin of which benzpyrene was applied for three months and in 5 of 8 mice painted for two months. Papilloma was noted in 7 of 8 animals treated with methylcholanthrene for three months and in 3 of 8 mice painted for two months and observed thereafter for thirty or forty-five days. No carcinoma was obtained in the methylcholanthrene-treated animals.

Wounds made in untreated skin showed a peak of mitotic activity in both the old marginal and the new regenerated epithelium five days after excision. After ten days the number of mitoses was normal

again. However, in the epidermis treated with carcinogens the number of mitoses remained high even after ten days of repair. In the present series the periods of observation were sufficiently long to allow complete epithelization of the defects.

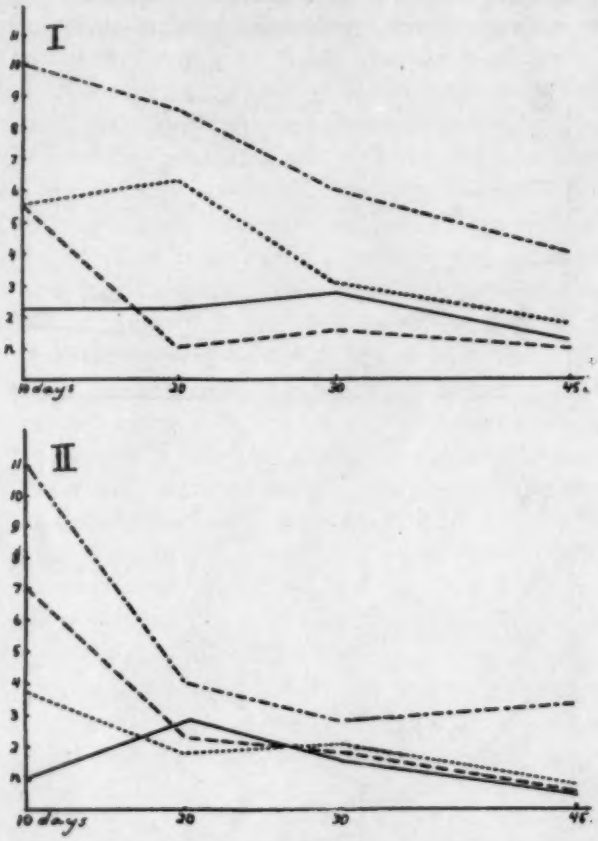

Chart 1.—Mitotic cycle in the old epithelium from the tenth to the forty-fifth day of wound repair in mice treated with 20-methylcholanthrene for two weeks (straight line), one month (broken line), two months (dotted line) or three months (dot and dash line); *n* stands for the normal value and the numbers above *n* for multiples of the normal.

Chart 2.—Mitotic cycle in the new regenerating epithelium from the tenth to the forty-fifth day of wound repair in mice treated with 20-methylcholanthrene for two weeks (straight line), one month (broken line), two months (dotted line) or three months (dot and dash line); *n* stands for the normal value and the numbers above *n* for multiples of the normal.

In charts 1 to 4 the mitotic counts made in the marginal and in the regenerated epithelium during later stages of wound healing are presented. Since in untreated animals the number of mitoses had

returned to normal after ten days, this period was chosen as the starting point of the present investigation and normal mice are not included in these charts. Excluded from the charts are, furthermore, those mice in which carcinoma had invaded the scars or was so close to them that mitotic counts of nontumorous epithelium could not be made near the regenerating epidermis. Among these animals were

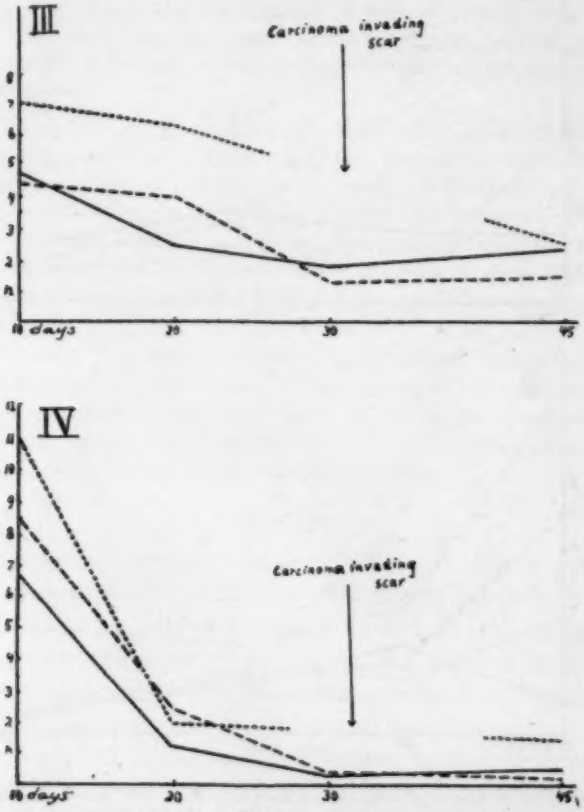

Chart 3.—Mitotic cycle in the old epithelium from the tenth to the forty-fifth day of wound repair in mice treated with 3,4-benzpyrene for two weeks (straight line), one month (broken line) or two months (dotted line); *n* stands for the normal values and the numbers above *n* for multiples of the normal.

Chart 4.—Mitotic cycle in the new regenerating epithelium from the tenth to the forty-fifth day of wound repair in mice treated with 3,4-benzpyrene for two weeks (straight line), one month (broken line) or two months (dotted line); *n* stands for the normal values and the numbers above *n* for multiples of the normal.

all those painted with benzpyrene for three months and 2 of 8 painted for two months. Mitotic counts could be obtained in and around the wounds in all mice painted with methylcholanthrene, without interference from the papillomatous growths. After periods of treat-

ment with either carcinogen ranging up to two months the mitotic counts of the marginal and the regenerated epithelium showed a tendency to return to normal values. The slight rises seen here and there are probably due to some irregularities beyond control either in the wound itself or in its location or in the local rhythmic variations of the mitoses in the epidermis. Thirty days subsequent to excision the mitotic counts dropped to levels ranging from three times the normal to below normal. Slowest in returning to lower values were, according to expectation, the mitotic counts following three months of painting with methylcholanthrene. They did not decline below four times the ordinary number. However, this constituted a considerable fall from their maximum of ten times the usual figures, seen after ten days of repair, and it corresponded in absolute values to the drop of from five and a half times the normal to normal after one month of painting. On the whole, conditions in the regenerated epithelium paralleled those in the marginal epithelium, although the mitotic activity declined more rapidly in the former. A sudden fall usually occurred between the tenth and the twentieth day after operation. After forty-five days, in most instances, the number of mitoses dropped even to below normal, thus indicating a particularly inactive epithelium. Only after methylcholanthrene had been applied three months or benzpyrene for two months were the counts above normal. This may well present merely a delayed return to normal.

The results of the present experiments confirm our previous observations concerning the effects of carcinogens on the course of repair in the skin of growing mice. The return of the mitotic counts to normal values was again delayed; a decline of the mitotic activity in both old and new epithelium was noted only after the defects were epithelized, that is, between the tenth and the twentieth day of healing. Thus, in accordance with the observations of Loeb,[3] the meeting of the epithelial tongues in the centers of the wounds resulted in an effective inhibition of the proliferation of the regenerating epithelium. This is thus true not only of the normal skin but also of the epidermis painted with carcinogens. What made the migrating epithelial cells overcome the forces that had inhibited their movement at earlier stages of wound healing is uncertain. Since the treatment with the carcinogen had been discontinued at the time of the making of the wound, the effect of each carcinogenic agent lessened progressively as the interval between excision and the date of killing increased. It is thus possible that the stimulus of the wounds after a while became

3. Loeb, L.: Arch. f. Entwcklngsmechan. d. Organ. **6**:297, 1898. Loeb, L., and Spain, K. C.: J. Exper. Med. **23**:107, 1916.

predominant over the effect of the carcinogen. Moreover, the regenerating epithelium was never exposed to the direct influence of the carcinogen, and this might be another reason that it behaved in a manner similar to the untreated epithelium. Experiments with cutaneous transplants are in progress which will further test these phenomena and in particular the possible role of the base of the wound in the migration of the epithelium.

With mitoses in the new epithelium at a nearly normal level, it is not surprising that tumors did not develop in the scars and that we did not find any correlation between wound healing and tumor formation. Single or multiple neoplasms developed in various places within or around the painted areas, near or at a distance from the line of excision. On the other hand, carcinomas originating at some distance from the wound often invaded and completely obliterated the regeneration areas secondarily. Failure of new growths to originate in healed wounds of mice has been reported also by MacKenzie and Rous [4] and Brunschwig, Tschetter and Pissell,[5] although the former investigators noticed tumor formation under similar conditions in the ears of rabbits. According to Mottram,[6] scarring did not influence the number of tumors in the skin of methylcholanthrene-treated mice; however, the appearance of these tumors was hastened. Pullinger [7] found the risk of a tumor developing in skin treated with benzpyrene increased by 13 per cent after a single surgical excision. Recently Lacassagne and Latarjet,[8] likewise studying the development of carcinoma in healed wounds, observed a retardation of the downgrowth of regenerating epidermal appendages in the dermis. These authors attributed the failure of tumors to develop in the scars to the absence therein of appendages, which usually show considerable mitotic activity. On the other hand, they correlated the appearance of tumors at the edges of the scars to the presence of hyperplastic hair follicles and sebaceous glands. However, there is at the margin of the defects a marked increase of mitotic activity of the epithelium irrespective of the appendages. This mitotic stimulation might, under certain conditions, play a part in localizing a tumor at the edge of a healed wound or cause a neoplasm to appear at an earlier date than it would in intact skin. But in our experiments there was no

4. MacKenzie, I., and Rous, P.: J. Exper. Med. **73**:391, 1941.

5. Brunschwig, A.; Tschetter, D., and Pissell, A. D.: Ann. Surg. **106**:1084, 1937.

6. Mottram, J. C.: J. Path. & Bact. **56**:391, 1944.

7. Pullinger, B. D.: J. Path. & Bact. **57**:467, 1945.

8. Lacassagne, A., and Latarjet, R.: Cancer Research **6**:183, 1946.

such stimulating effect; there was, on the contrary, a definite trend of the epithelium toward a return to a resting state.

SUMMARY

The inhibition of the epithelization of wounds made in the skins of young mice treated with 3, 4-benzpyrene or 20-methylcholanthrene for two or three months previous to the making of the defects is temporary. The regenerating epithelium overcomes the forces opposing its migration as it advances into the wound at the end of the second or the beginning of the third week of healing. Coinciding with the epithelization of the defect is a fall in the mitotic activity in both the old marginal and the new regenerated epithelium. This tendency of the epithelium to return to a resting state is considered the reason that, under the present experimental conditions, no correlation was noted between the former site of the wound and the place of tumor formation.

DISSEMINATED RETICULOENDOTHELIAL TUMOR OF THE BONE MARROW WITH NODULAR OSTEOSCLEROSIS

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IN THE CASE to be described a condition was presented which is closely related to reticuloendotheliosis, or aleukemic reticuloendotheliosis, or systemic hyperplasia of the reticuloendothelial cells, but which is different in some respects. It is not a diffuse disorder, whereas the others are. It is limited to a number of circumscribed areas of the bone marrow and is associated with marked bone formation that is likewise limited to the affected areas. The fact that the disease process is not generalized but disseminated and multiple and that the lesion is a true neoplasm of reticuloendothelial cells of the marrow would warrant its classification as multiple reticuloendothelial myeloma; however, the name "myeloma" denominates a well characterized disease associated not only with the proliferation of cells of the marrow but also with profound disturbances of the protein metabolism. These were not displayed in the present observation.

REPORT OF A CASE

A 60 year old white American woman was admitted to Stanford University Hospital for observation of hypertensive disease (blood pressure 200 systolic and 120 diastolic) of about a year's duration, during which time she had lost some weight. The heart was slightly enlarged. The liver and the spleen were of normal size. No lymph nodes were palpated. No masses or other signs of tumor were detected.

Roentgen Examination.—Excretory urography carried out in an attempt to determine the cause of hypertension revealed no abnormalities of the urinary tract. Plain roentgenograms disclosed numerous rounded sclerotic densities of various sizes in the innominate bones, the sacrum, the lumbar vertebrae, the ribs and the calvarium. The fifth lumbar vertebra was entirely dense.

The nature of these densities was not apparent. The possibility of bone-forming tumor metastases or of Hodgkin's disease with atypical skeletal distribution was considered.

Laboratory Examination.—The red blood cell count was 4,360,000; the hemoglobin content, 78 per cent (Sahli); the color index, 90. The leukocyte count was 14,000. The total neutrophil percentage was 80; 20 per cent were banded and 60 per cent segmented. Eosinophils were 4 per cent; basophils, 1 per cent; lymphocytes, 11 per cent; macrocytes, 4 per cent. The platelet count was 401,120. Of the erythrocytes, 0.7 per cent were reticulocytes. The hematocrit reading was 36.5

From the Department of Radiology, Stanford University School of Medicine.

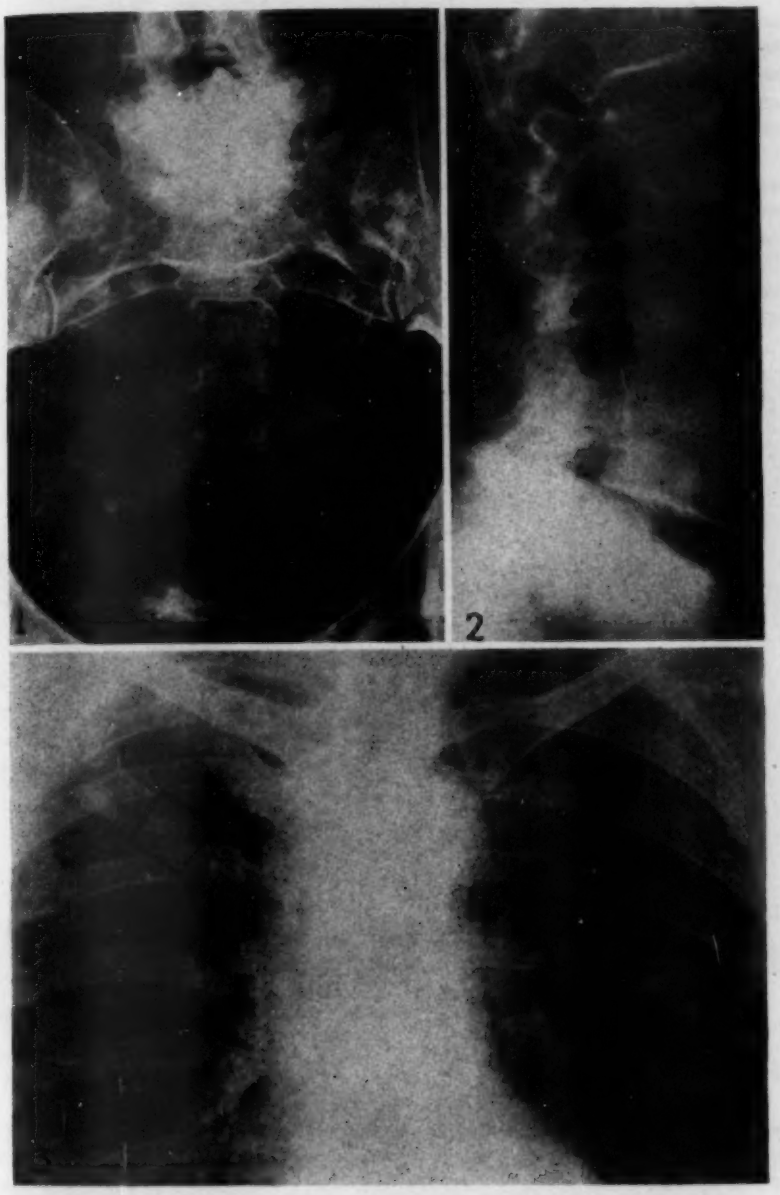

Fig. 1.—Dorsal view of the sacroiliac area. Note the outstanding density of the body of the fifth lumbar vertebra and of adjacent parts of the sacral bone. Numerous rounded densities may be seen in the sacral wings and in the innominate and pubic bones.

Fig. 2.—Left lateral view of the lumbar portion of the spine. The bodies of the second and fourth lumbar vertebrae show several rounded densities. The body of the fifth lumbar vertebra is dense in its whole extent.

Fig. 3.—Anterior view of the chest. The anterior portions of the second and third ribs on the right and the third rib on the left reveal a dense area. Bone was taken from the distal end of the second rib on the right for biopsy.

per cent. The red cells varied in size and color but not in shape. The leukocytes showed no abnormality. The platelets were numerous and apparently normal from the standpoint of morphology. Marrow obtained by sternal puncture showed an increased number of plasma cells of the normal (Marschalko) type and an increased number of megakaryocytes. No neoplastic cells were present.

Further tests revealed high serum chlorides (6.83 mg. per hundred cubic centimeters), high serum inorganic phosphates (4.9 mg. per hundred cubic centimeters) and a high basal metabolic rate (+35 to 37). All other values were within normal limits. No Bence Jones protein was detected. Serum albumin amounted to 3.8 Gm. and globulin to 2.7 Gm. per hundred cubic centimeters. The albumin-globulin ratio was 1.407 (normal); the serum protein was 6.87 mg. per hundred cubic centimeters.

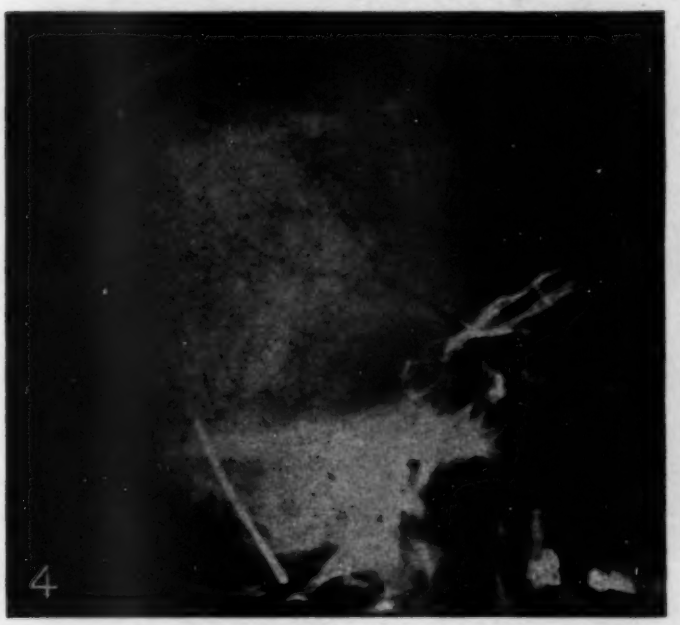

Fig. 4.—Irregular densities, some minute and some larger, are seen in the bones of the cranial vault. The parietal bones are mainly affected.

Fig. 5.—Roentgenogram of the biopsy specimen taken from the distal end of the second rib on the right, showing a circumscribed area of bone density.

Since the results of laboratory tests, physical examination and clinical observation failed to divulge any sign of a primary tumor, and since no explanation for the striking roentgenologic findings could be given, biopsy of bone was done and reported on as follows (Dr. William H. Carnes, of the department of pathology of Stanford University School of Medicine): "The interstices between the bone trabeculae were filled with a fine but distinct collagenous stroma and abundant reticulum. The cells varied somewhat in size as well as shape. The nuclei were medium sized and often eccentric and were composed of a dark-staining coarse chromatin, which was frequently distributed in a peripheral or a radial pattern, with identifiable nucleoli. No mitoses were seen. The cytoplasm was abundant, with relatively basophilic staining and frequently a distinct peri-

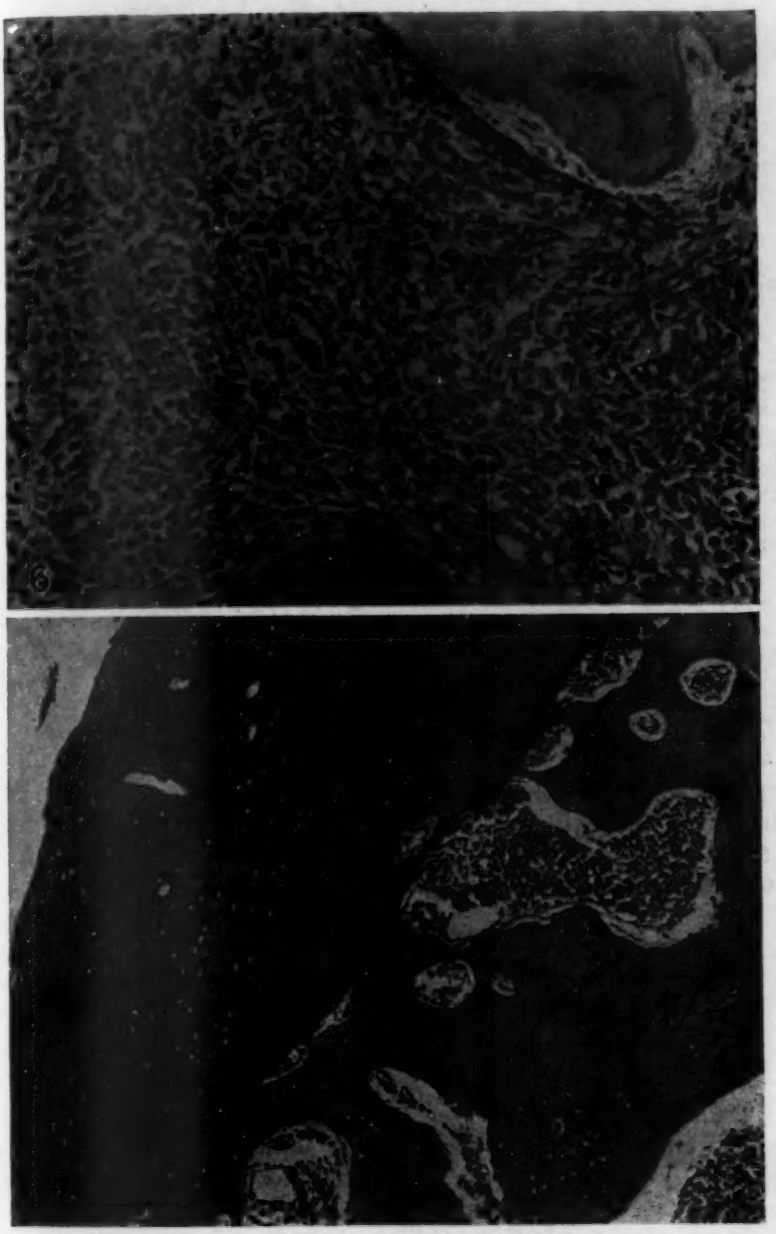

Fig. 6 (case 1).—Photomicrograph (× 189) showing tumor cells of irregular shapes and sizes, with abundant cytoplasm and dark-staining small nuclei. Note the large amount of intercellular stroma. There is lacunar erosion of a bone trabeculum at the lower end of the picture. Hematoxylin-eosin.

Fig. 7.—Photomicrograph (× 141.5) showing massive new formation of bone tissue with dense lamellated bone structures replacing the cortex (at the left and the middle of the picture). Newly formed trabeculae of irregular distribution (almost mosaic-shaped lamellas) may be seen at the left. Note the tumor tissue in haversian canaliculae and in marrow spaces. Hematoxylin-eosin.

nuclear clear zone. Occasionally, the cytoplasm contained a few fine vacuoles, especially at the cell margin. A few characteristic large round cytoplasmic inclusions were seen, indistinguishable from Russell's fuchsin bodies. This cellular proliferation corresponded closely to the bone hyperplasia in all sections and extended only a short distance beyond it. Outside the lesions, the bony trabeculae, the cortex and the marrow were normal. The neoplastic nature of the lesion was agreed on by all who examined the slides; however, the cells were not typical myeloma cells, and the abundance of stroma, reticulum and new bone formation was striking."

In view of the microscopic appearance of the tumor, showing a large amount of reticulum and collagenous stroma, and the resemblance of its cellular elements to endothelium, with signs of phagocytosis and vacuolation, it was stated to be reticuloendothelial in origin.

The patient was discharged when diagnostic procedures had been completed. She was readmitted two months later with signs of cardiac decompensation. The liver was now somewhat enlarged (congested). Repeated and additional laboratory studies provided no further information. Hepatolienography with intravenous injection of a colloidal suspension of thorium dioxide showed normal and homogeneous phagocytosis of the thorium dioxide on the part of the reticuloendothelium of the liver and the spleen.

After discharge the patient's condition steadily declined, and she died with signs of congestive failure eight months after her first admission. Permission for postmortem examination was refused.

COMMENT

Histologic examination proved that we were dealing with a disseminated neoplastic lesion of the bone marrow of reticuloendothelial origin. Whether or not other organs rich in reticuloendothelium participated in the disease process was impossible to determine without autopsy; however, none of the clinical or laboratory findings indicated the presence of lesions in internal organs. The only evidence of foci of disease existed in the marrow of spongy bones, ribs, the calvarium, the spinal column and the pelvis. These are the bones in which multiple myeloma commonly occurs.

Roentgenologically, the distinguishing feature of this case was the conspicuous bone formation within areas occupied by the tumor. Microscopic examination showed that this bone tissue was formed in various ways. Dense lamellated bone was deposited on the surface of the cortex by reason of periosteal apposition of bone tissue in the tumor area. In many places this newly formed bone replaced the entire cortex, indicating that previous destruction was followed by regeneration. Irregular, massive, nonlamellated bone trabeculae intruded at identical locations into the marrow cavity. In other areas it was evident that reticular and fibrous interstices of the tumor were submerged into the newly formed nonlamellated bone spicules, indicating that these originated as bony metaplasia of the reticular stroma of the tumor. The distribution of the bone lamellas did not follow the rules of statics.

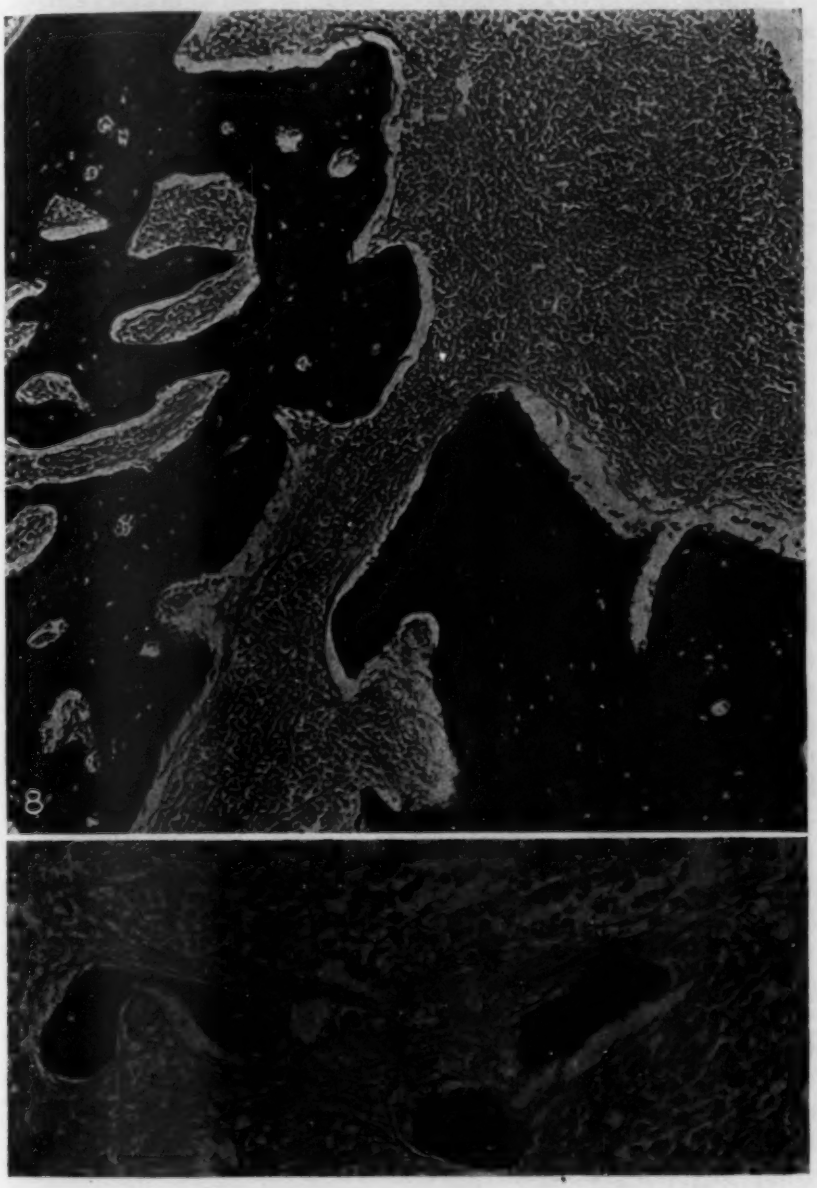

Fig. 8.—Photomicrograph (× 80) showing the distribution of newly formed trabeculae within the tumor. The old cortex is seen in the left upper corner. New trabeculae extend from the medullary surface of the cortex into the tumor tissue. Note the increase of reticular interstices of the tumor at the inner surface of the newly formed trabeculae. Tumor tissue entirely replaces the marrow.

Fig. 9.—Photomicrograph (× 200) showing metaplastic new formation of non-lamellated bone trabeculae within the fibrous reticulum of the tumor. Van Gieson stain.

Hyperplasias of reticuloendothelial tissue, as well as primary tumors, are generally considered as bone-destroying lesions. Osteogenesis observed in such disorders has been thought to be a reaction to the osteogenic impulses occurring in the tumor or a sign of reparative tendencies on the part of the endosteum or the periosteum. To date, active bone formation as a specific property of these tumors has not been recorded because osteogenesis was mostly obscured by bone destruction due to the overwhelming destructive properties of these tumor cells. It will be shown later, however, that detailed roentgenologic and histologic investigations almost always reveal bone formation in reticuloendothelial tumors in addition to bone destruction if a sufficient amount of collagen or reticulum participates in the formation of the tumor. This is most frequent in late stages of the disease.

Cases like the present one are rare not only with regard to the excessive bone formation but also with regard to the nature of the underlying disease.

We are well aware of the fact that without autopsy no definite statement can be made concerning possible participation of internal organs in the disease process. The striking findings, however, necessitate the publication of this case, specifically because a review of references yielded no information concerning similar observations. Diffuse reticuloendothelial hyperplasias of internal organs and of the marrow, as well as solitary tumors of the same origin, are well known. Disseminated nodular distribution of the foci of the disease has apparently not been recorded without coexisting lipoid granulomatosis.

The often cited case of Marckwald [1] in which multiple endothelial tumor of the bone marrow with bone formation was observed is not clearly understood. From the data and the illustrations given I was not convinced of the reticuloendothelial origin of the tumor. Chester [2] reported a case of lipoid granulomatosis with circumscribed sclerosis of several bones. Microscopic examination revealed that bone had been deposited in fibrous interstices of cellular hyperplasias. The deposits were present in abundance in places where the disease displayed signs of having healed spontaneously. Wassiljeff [3] observed a case in which diffuse osteosclerosis of the skeleton and diffuse reticuloendothelial hyperplasia of the marrow were present. The latter case belongs to the group in which generalized osteosclerosis is associated with disorders of blood formation.

1. Marckwald, W. L.: Virchows Arch. f. path. Anat. **141**:128, 1895.
2. Chester, W.: Virchows Arch. f. path. Anat. **279**:561, 1930.
3. Wassiljeff, G., in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 4.

SUMMARY

A case of a primary disseminated nodular reticuloendothelial tumor of the bone marrow with neoplastic growth of both the reticular and the endothelial components of this cellular system has been observed. Marked circumscribed bone formation was present in areas involved by the tumor. The newly formed bone tissue was mainly contributed by metaplasia of collagenous and reticular interstices of the tumor itself. Roentgen findings were suggestive of osteoblastic tumor metastases.

Multiple primary bone-forming reticuloendothelial tumor of the marrow is an extremely rare disease. I failed to discover any reference to a similar case in the literature at my disposal.

ALLOXANTIN

An Investigation of the Substance as Used in Experimental Production of Diabetes

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JOHN S. WATSON, M.D.

AND

ELLA PERKINS, M.Sc.

PHILADELPHIA

IN 1937 JACOBS [1] discovered that alloxan injected into normal fasting rabbits produced hypoglycemia and convulsions. To ascertain whether this action was due to alloxan itself or to one of its derivatives, he tested 12 other closely related substances, with negative results. Among the drugs was alloxantin. These negative observations were confirmed in dogs by Goldner and Gomori [2] in 1944. In the same year, however, Koref and associates [3] reported that alloxantin injected intravenously into rabbits had an effect identical with that of alloxan. There was temporary hyperglycemia followed by marked hypoglycemia, then permanent hyperglycemia. The histologic changes in the islets of Langerhans consisted of degeneration and necrosis with diminution in the number of beta cells. They further reported that, despite low blood sugar levels, none of their animals had hypoglycemic convulsions. If this were true, alloxantin might prove to be a good substitute for alloxan, because the latter, while its diabetogenic action is undisputed, has the disadvantages that in spite of close vigilance some of the animals succumb to hypoglycemic convulsions and, of those that survive, many die within the next few days from hepatic and renal necrosis.[4] It thus seemed desirable to reinvestigate the diabetogenic action of alloxantin.

PROCEDURE

Thirty-five rabbits weighing between 2,000 and 3,020 Gm. were given injections of alloxantin in the following manner: Six were given 1,000 mg. each subcutaneously; 3 were given 800 mg. each and 2 were given 1,000 mg. each intraperitoneally; 6 received 100 mg., 15 received 150 mg. and 3 received 200 mg. per kilogram of body weight intravenously. A week later 3 of the rabbits given subcutaneous injections received intravenously 200 mg. of alloxantin per kilogram

From the Clinical Laboratories, Jefferson Medical College Hospital.

1. Jacobs, H. R.: Proc. Soc. Exper. Biol. & Med. **37**:407, 1937.
2. Goldner, M. G., and Gomori, G.: Endocrinology **35**:241, 1944.
3. Koref, O.; Vargos, L.; Rodriguez, F. H., and Telchi, A.: Endocrinology **35**:391, 1944.
4. Herbut, P. A.; Watson, J. S., and Perkins, E.: Hepatic and Renal Necrosis in Alloxan Diabetes in Rabbits, Arch. Path. **41**:516, 1946.

of body weight, and 3 of those given intravenous injections of 100 mg. per kilogram of body weight and 5 of those that had received intravenously 150 mg. per kilogram of body weight were each given a second injection of 150 mg. of alloxantin per kilogram of body weight. The levels of blood sugar and blood nonprotein nitrogen were determined before the injection, and repeated determinations of blood sugar levels were made at approximately hourly intervals on many of the rabbits for the first twelve hours after the injection and on all the rabbits at irregular intervals during the two weeks of the experiment. Blood sugar, blood nonprotein nitrogen and blood cholesterol levels were determined on all the animals at the time of death. In the case of rabbits with permanently elevated blood sugar the urine was tested for sugar and acetone bodies. All surviving animals were killed in two weeks with a blow on the head. Immediately after death, tissues from the lungs, the liver, the kidneys, the adrenal glands and the pancreas were fixed in solution of formaldehyde U. S. P. diluted 1:10 and in Helly's fluid,[5] and sections of these were stained with hematoxylin and eosin. In addition, sections of the pancreas were stained by Bensley's method for alpha and beta granules.

Alloxantin is soluble in water or in saline solution in very small amounts. Heating a 1 per cent, 2.5 per cent or 5 per cent solution in water or in saline solution to 50 C. did not increase the solubility appreciably. The relatively large crystals of alloxantin were therefore ground to a fine powder in a sterile mortar and the amount allotted to each rabbit was weighed separately and put into a sterile test tube. About 10 cc. of sterile saline solution was added to each tube just before injection, and the consequent suspension was given relatively slowly. Since alloxantin precipitates out rapidly, it was necessary to turn the syringe continually while the drug was being introduced. In spite of this some of the powder precipitated out, and this was resuspended in 10 cc. of saline solution and injected as before. To avoid blocking, we used a 16 gage needle for the subcutaneous and intraperitoneal injections and an 18 gage one for the intravenous injections.

RESULTS

Clinical Observations.—Alloxantin injected subcutaneously had no clinical effect on the rabbits. If the drug was irritating, none of the animals cried or showed other signs of discomfort.

A few seconds after intraperitoneal injection of the drug the rabbits became stretched out, with their heads arched backward, and almost simultaneously underwent twitchings of the muscles of the back, the abdomen and the lower extremities. Two of these animals recovered, and 3 died in from five to fourteen hours after the injection.

Intravenous administration did not prove as irritating as anticipated, for only 2 of the rabbits cried, and that appeared to be more from fear than from pain. All animals threshed about a little but not more than those which received alloxan. The veins that were used, however, became completely thrombosed a few seconds after the injection was completed. Of the 6 animals that received 100 mg. per kilogram of body weight, 1 died during the injection and another three hours later. The rest showed no ill effects. Seven of the 15 rabbits that received 150 mg. of alloxantin per kilogram of body weight died from a few minutes to nineteen hours after the injection. Most of those that survived the immediate effects but died

5. Helly's fluid is a modification of Zenker's solution in which, instead of 5 cc. of glacial acetic acid, solution of formaldehyde U. S. P. is used in the concentration of 5 per cent.

later merely became drowsy and listless and just before dying showed some jerky purposeless movements. One animal that died nine hours after the injection had severe pulmonary edema, with frothy pink fluid literally dripping from the nose and the mouth. In 2 rabbits typical hypoglycemic convulsions developed two hours and eighteen hours after the injection, which were readily controlled with subcutaneous injections of dextrose. Later permanent hyperglycemia developed in these animals. Two of the 3 rabbits that received an initial dose of 200 mg. of alloxantin per kilogram of body weight died within five minutes after the injection, and 1 was found dead approximately four hours later.

The reactions to the second injections were similar to those following the first. One of the 8 rabbits that received 150 Gm. per kilogram of body weight showed twitchings, crying and exuding of frothy fluid from the nose and the mouth five hours after the injection, and died shortly thereafter. The remaining 7 animals in this group showed no ill effects. One of the 3 rabbits that received a second injection of alloxantin in a dose equivalent to 200 mg. per kilogram of body weight died at once; the others survived. Diabetes did not develop in any of the animals that received a second injection.

Chemical Observations.—Followng the initial injection there was transitory hyperglycemia followed by temporary hypoglycemia, then either transitory hyperglycemia with a return to normal in a few days or, in 2 rabbits, permanent hyperglycemia with excretion of sugar in the urine. The normal blood sugar levels varied from 70 mg. to 102 mg., with an average of 91 mg., per hundred cubic centimeters. In forty minutes to five hours the levels rose to from 123 mg. to 400 mg., with an average of 202 mg., per hundred cubic centimeters. The low blood sugar levels were reached in from five to fifteen hours and varied from 36 mg. to 80 mg., with an average of 69 mg., per hundred cubic centimeters. The subsequent high levels were reached in from five to twenty hours and varied from 114 mg. to 363 mg. per hundred cubic centimeters. The blood sugar levels following second injections were not determined until twenty-four hours after the administration of alloxantin, at which time they ranged from 129 mg. to 448 mg., with an average of 195 mg., per hundred cubic centimeters. After twenty-four hours the levels gradually declined until, within a week, they were all normal. The normal level of blood nonprotein nitrogen varied from 36 mg. to 64 mg. per hundred cubic centimeters. It remained unchanged in all but 2 rabbits. In one of these, which died twenty-four hours after the first injection, the level was 175 mg., and in the other, which died five hours after the second injection, it was 184 mg., per hundred cubic centimeters. The level of blood cholesterol was normal in all animals.

Gross Pathologic Observations.—In 4 of the 6 rabbits that received alloxantin subcutaneously an abscess developed at the site of injection. The largest abscess measured 10 cm. across, but the walls were collapsed, and there was only a little pus present; the overlying skin was a little stiff but showed no gangrene. One animal had a perinephric abscess on the right side, which was apparently directly continuous with the subcutaneous abscess. Except for slight accentuation of the lobular markings of the liver in 2 other rabbits, no other organic involvements were observed in this group.

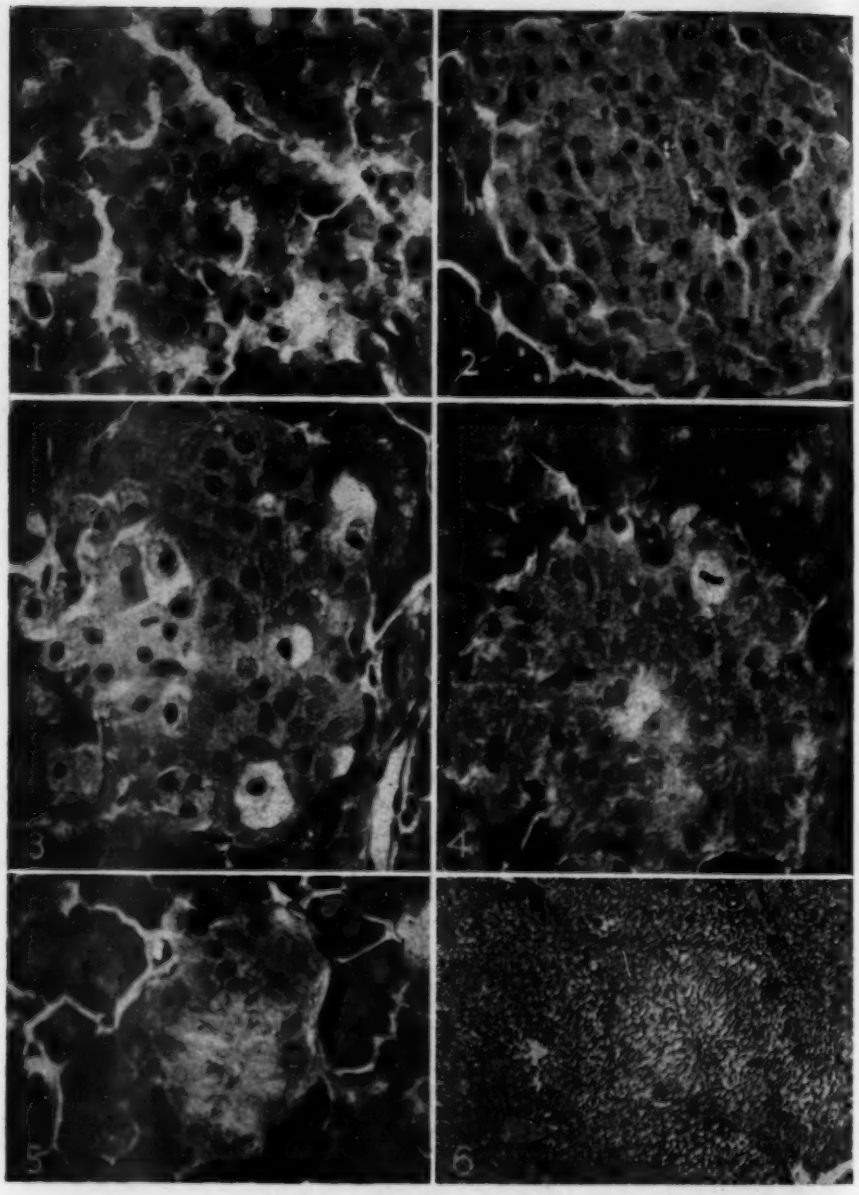
The 2 of the 5 rabbits receiving intraperitoneal injections that died within five hours disclosed general peritonitis. There was about 150 cc. of fluid, together with dense but recent fibrinous adhesions of loops of the intestines. The rabbit that died at fourteen hours showed excess fluid but no adhesions. Of the 2 that survived and were killed in two weeks, one revealed fibrous adhesions between the liver and the right kidney, and the other was normal.

There were no gross pathologic changes in the 2 rabbits that died during the intravenous injection of the drug. In 10 animals that died within a few minutes to twenty-two hours after the intravenous injection there were severe pulmonary congestion, edema, hemorrhages, atelectasis and emphysema. As already mentioned, in 2 of the rabbits this was so severe that frothy pink fluid dripped freely from the nose and the mouth. In 3 animals that received 200 mg. of alloxantin per kilogram of body weight the lungs actually appeared as if they were scorched. They were normal in 1 rabbit that died twenty-three hours after the injection and in all rabbits that were killed at the end of two weeks. The lobular markings of the liver were exaggerated in 3 animals that died seven, nine and twenty-three hours after the injection, but none showed hepatic necrosis. The liver was normal in all the other rabbits of this group. There were petechiae in the kidneys of 1 animal that died three hours after the injection and marked swelling and mottling of the cortex in another that died in twenty-two hours. The remaining organs showed no noteworthy changes.

Microscopic Observations.—There were histologic changes in the islets of Langerhans in 11 of the 35 rabbits. Three of these were rabbits given intraperitoneal and 8 were rabbits given intravenous injections. The initial alterations were seen as early as forty minutes after the injection and consisted of pyknosis of a few cells with no visible change in the cytoplasm. The distribution of these cells varied. Sometimes they were scattered throughout the islets; at other times they appeared to involve only the central portion (figs. 1 and 2). Within three hours after the injection some of the islets showed pyknosis of almost all the cells while others were less severely involved. By this time the cytoplasm was somewhat swollen and more dense. At fourteen hours the degenerating cells were definitely swollen and the cytoplasm granular, and by twenty-two hours the cells in the central portion showed severe degeneration progressing to complete necrosis. The nuclei were still pyknotic but were less heavily stained, or they disappeared entirely, and the cytoplasm was granular or vacuolated (fig. 3). Often only a thin ring of cells filled with alpha granules was found at the periphery. In such islets beta granules were not apparent. They were, however, still present in those islets showing only degenerative changes. In one of the 2 rabbits in which diabetes developed and which were killed at the end of two weeks the islets were fewer, but in the other they were not. In each, however, they were reduced to about one quarter of the normal size, and the central portions of many of them were devoid of cells and filled with granular debris (fig. 5). The cells at the periphery were normal and contained alpha granules. One mitotic figure was noted in a hypertrophied islet in one of these rabbits (fig. 4). The same islet disclosed two tiny foci of detritus indicating that it had been previously damaged.

The lungs of the animals that died during the injection showed no changes except some atelectasis. All others that died within the first day showed moderate to severe congestion, edema, hemorrhages and varying degrees of atelectasis and emphysema. The vessels, however, disclosed no microscopic alterations. The lungs of an animal that died with pulmonary edema five hours after the second injection showed in addition foci of old pneumonia and thrombosis of the veins. The former consisted of plugs of fibrin surrounded by mononuclear cells. The septums were broad, and a few contained neutrophils and eosinophils. In these areas there were several medium-sized and small veins that were plugged with an admixture of fibrin and leukocytes. The arteries were not affected.

The livers of 5 rabbits disclosed in the central areas of the lobules varying degrees of degeneration advancing to complete necrosis. In one of these animals, dying at fourteen hours, the involvement was small, was located to one side of



(See legends on opposite page)

the central vein and consisted of simple degeneration progressing to necrosis of the liver cells with slight erythrocytic extravasation. In the remaining 4 animals, which died seven hours, nine hours, twenty-two hours and fourteen days after the injection, the lesion occupied as much as three fourths of the lobule and completely surrounded the central vein (fig. 6). In some areas leukocytic extravasation was scanty; in others it was profuse. In all cases the hepatic cells immediately adjacent to the portal triads were intact. In the livers of the other 30 rabbits there were no significant alterations.

In only 1 animal were the kidneys abnormal. This rabbit received a single intravenous injection of alloxantin in a dose equivalent to 150 mg. per kilogram of body weight. He died about twenty-two hours after the injection. Grossly, as already mentioned, the kidneys showed swelling and mottling of the cortices. Microscopically, this mottling was seen to consist of areas of severe degeneration and swelling of the renal tubules alternating with areas of dilatation of the lumens and sloughing of the tubular epithelium. These changes were practically confined to the proximal convoluted tubules. The lumens of the more distal portions of the nephrons contained granular and erythrocytic casts. All the glomeruli were swollen and contained focal areas of necrosis. The interstitial tissue disclosed edema and foci of detritus, nuclear fragments and leukocytes. The vessels were normal. The adrenal glands were normal.

COMMENT

Koref and associates [3] reported that since alloxantin oxidizes readily it was kept in a vacuum until used. Perhaps this was due to their method of preparing the drug, for the commercially available alloxantin is supplied in an ordinary unsealed bottle with no precautions to prevent oxidation. Once the substance is in solution and exposed to air, oxidation as evidenced by a change to a pink or a red color occurs. Even with such exposure, however, the oxidation of alloxantin is not any more rapid that that of alloxan.

There is apparently no disagreement as to the irritating action of alloxantin. Although none of our rabbits that received subcutaneous or intravenous injections manifested any acute pain or violent general reaction, the aural vein used became thrombosed immediately on the completion of the injection. Intraperitoneally, too, the drug must have been irritating, for the animals stretched out to a maximum and underwent twitchings and even contractions of the muscles of the abdomen, back and the lower extremities. The 3 rabbits that died showed excess peritoneal fluid and 2 disclosed in addition extensive adhesive

Explanation of Figures

Fig. 1.—A portion of an islet of Langerhans with extreme pyknosis of most of the nuclei and condensation of the cytoplasm, in a section of pancreas from a rabbit that died four hours after intravenous injection of alloxantin. Hematoxylin and eosin; × 400.

Fig. 2.—An islet showing extensive degeneration of all the central cells with beginning fading of the nuclei, in a section of pancreas from a rabbit that died twenty-three hours after intravenous injection of the drug. At the periphery of the islet there is an intact rim of alpha cells. Hematoxylin and eosin; × 400.

Fig. 3.—An islet showing several cells with pyknotic nuclei and granular cytoplasm. In 2 of the cells the cytoplasm also shows hydropic degeneration. The rabbit died twenty-two hours after intravenous injection of the drug. Hematoxylin and eosin; × 400.

Fig. 4.—A relatively hypertrophied islet wherein one cell is in mitosis. Note the two tiny foci of debris indicating the sites of previous necrosis. The rabbit had diabetes. Hematoxylin and eosin; × 400.

Fig. 5.—A shrunken islet of a rabbit with diabetes. Note the intact peripheral cells and the large central area of detritus. Hematoxylin and eosin; × 400.

Fig. 6.—Section of the liver of a rabbit that was killed two weeks after it had received an injection of alloxantin; the section shows complete necrosis of the central portion of a lobule. Hematoxylin and eosin; × 50.

peritonitis. At the end of two weeks fibrous peritoneal adhesions were present in 1 of 2 surviving rabbits. A third indication of the irritating action of alloxantin, apparently on the capillaries, was the pulmonary edema that developed in all animals in which alloxantin was injected intravenously and which died early. In 2 of these it was so severe that terminally frothy pink fluid dripped freely from the nose and the mouth.

Repeated determination of the blood sugar levels of many animals revealed a curve identical with that obtained with alloxan except that the deviations from the normal were somewhat less pronounced. There was temporary hyperglycemia followed by temporary hypoglycemia, then either permanent or transitory hyperglycemia. Koref [5] stated that in spite of low blood sugar levels none of their rabbits had convulsions. This we could not corroborate, for in our series there were only 2 rabbits in which diabetes developed, and each of these underwent convulsions.

The histologic changes in the islets of Langerhans were similar to, but much less extensive than, those produced by alloxan. Early there were pyknosis of the nuclei and condensation of the cytoplasm of a few or of many of the cells. In the central portions of some of the islets the nuclei gradually disappeared and the cytoplasm became granular, vacuolated and finally entirely desintegrated, leaving foci of complete necrosis. Unlike what was noted with alloxan, however, there was always a peripheral rim of normal alpha cells remaining, and in none of the rabbits was there complete necrosis of all the cells. In the animals in which permanent diabetes developed, the islets were reduced in size but not in number, and showed normal peripheral cells; some islets contained tiny central foci of detritus, which indicated areas of previous necrosis. Hyalinization of islet cells was not seen, but one mitotic figure was encountered in a hypertrophied islet. Since in this same islet there were two tiny foci of detritus, it seems fair to assume that the islet was the seat of necrosis and that its hypertrophy was due to active proliferation of the islet cells themselves. The changes in alloxantin-treated animals might, therefore, be more comparable to those seen in human diabetes than are the lesions produced by alloxan, for in man necrosis of the islets of Langerhans has rarely been observed [6]; vacuolation of the islet cells has occasionally been noted, and the absence of any histologic changes is common.[7] Thus it may be that alloxantin is more akin to the diabetogenic factor in man than is alloxan.

The side effects of alloxantin are somewhat different from those of alloxan. Severe pulmonary congestion and edema appears to play

6. Duffy, E.: J. Path. & Bact. **57**:199, 1945.

7. Warren, S.: The Pathology of Diabetes Mellitus, ed. 2, Philadelphia, Lea & Febiger, 1938, p. 31.

an important role in alloxantin-treated animals and account for most of the early deaths. Some indication of more permanent damage of the lungs is evidenced by the finding in 1 animal of foci of organizing pneumonia and thrombosis of some of the veins. Alloxan, on the other hand, produced no pulmonary changes. The damage done to the liver by alloxantin was less frequent, less severe and of a different distribution as compared with that produced by alloxan. The latter caused necrosis, regeneration and fibrous tissue replacement of the peripheral portions of the lobules, whereas the former produced degeneration and necrosis of only the central portions. Only 1 of the animals treated with alloxantin disclosed damage of the kidneys, while alloxan produced extensive degeneration and necrosis of the tubular epithelium in many rabbits.

An evaluation of the two methods of producing experimental diabetes indicates that the use of alloxan is superior to that of alloxantin for the following reasons: Alloxan is easier to administer in that it is more readily soluble in water and isotonic solution of sodium chloride. It is less irritating, and its action is more dependable; that is, in adequate doses it will produce diabetes in 100 per cent of the animals, whereas alloxantin in nonlethal doses will produce diabetes in only a few. Undesirable side effects causing death in some of the animals occur with both and, while the sites of these actions vary with each substance, they are all equally fatal, leaving little to choose between the drugs from this standpoint.

SUMMARY

Alloxantin suspended in isotonic solution of sodium chloride was injected into rabbits subcutaneously, intraperitoneally and intravenously. Administered by the intravenous route it produced an effect similar to that of alloxan. There was temporary hyperglycemia followed by temporary hypoglycemia, then either transient hyperglycemia or, in a few of the animals, permanent hyperglycemia with excretion of sugar in the urine.

Histologic changes in the islets of Langerhans consisted of pyknosis of the nuclei, granular and hydropic degeneration of the cytoplasm and ultimately complete necrosis of the cells in the central areas. In the animals in which diabetes developed, the islets were decreased in size but not in number, and some showed remaining foci of detritus. Undesirable side effects consisted of thrombosis of the veins of the ears, severe pulmonary congestion and edema, and necrosis of the central portions of the hepatic lobules.

Although the diabetogenic action of alloxantin is unequivocal, it is not a good substitue for alloxan because it is difficult to administer, it is toxic, and nonlethal doses will produce diabetes in only a few animals.

REACTION OF RATS FOLLOWING INJECTION OF ANTI-RAT-HEART IMMUNE SERUM

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THE role of immune and allergic reactions in the genesis of rheumatic fever has been studied by many investigators. In most of the experiments on animals a protein or a bacterial antigen has been injected that causes a variable circulating antibody response and also frequently an unknown degree of hypersensitivity of tissues. The experiments which will be presented were designed to produce an antigen-antibody reaction in which a specific antibody is supplied artificially in known amounts in order to minimize the number of experimental variables. Perhaps by this method lesions similar to those found in rheumatic fever can be produced.

Since rheumatic fever is a disease involving chiefly connective tissue, in the present investigation it was decided to prepare an antibody to rat connective tissue and then inject this into otherwise normal rats. Because there is considerable variation in the structure of connective tissue in various organs, the hearts of rats were used as a source of connective tissue since it is the connective tissue of the heart that is significantly involved in rheumatic fever.

Longcope [1] inoculated animals (rabbits, dogs, cats and guinea pigs) intravenously, intraperitoneally or subcutaneously with egg white or horse serum or both and later gave intoxicating doses of the same material. He found swelling of the muscle fibers and focal infiltrations of lymphocytes in the hearts of the rabbits. The lungs of guinea pigs had small patches like bronchopneumonia with peribronchial infiltrations rich in eosinophil leukocytes. The livers of the rabbits and the cats had regions of necrosis, often periportal. There were also changes in the kidneys. Smadel,[2] Smadel and Farr [3] and Swift and Smadel [4] produced nephritis in rats by injections of antiserums to rat kidneys. They noted changes in the hearts of some of their animals. They also injected rat heart antiserums into rats as a

1. Longcope, W. T.: J. Exper. Med. **18**:678, 1913; Arch. Int. Med. **15**:1079, 1915.

2. Smadel, J. E.: (*a*) J. Exper. Med. **64**:921, 1936; (*b*) **65**:541, 1937.

3. Smadel, J. E., and Farr, L. E.: J. Exper. Med. **65**:527, 1937.

4. Swift, H. F., and Smadel, J. E.: J Exper. Med. **65**:557, 1937.

control procedure but did not pay particular attention to the hearts of these animals because they were interested in renal changes. Rich and Gregory [5a] injected horse serum intravenously into rabbits and found lesions resembling periarteritis nodosa. In some rabbits they found cardiac lesions of the specific rheumatic type with Aschoff bodies and valvular changes.[5b, c] They pointed out that the tissue changes were the result of an anaphylactic type of hypersensitivity in contrast to the tuberculin type. As further evidence of the anaphylactic origin of the lesions of acute rheumatic fever, these observers [5d] mentioned the basic similarity of the pneumonitis caused by sulfonamide hypersensitivity and that occurring in rheumatic fever.

MATERIAL AND METHODS

Preparation of Rat Heart Antiserum.—The procedure was similar to that employed by Smadel [3] in the preparation of antikidney serum. With the rat under ether anesthesia, the heart was perfused with saline solution through the inferior vena cava. A perforation in an artery of the neck permitted the escape of blood and saline solution. In some instances the heart was removed while still beating and placed in saline solution until movement stopped. Each heart was then cut into small pieces, and these were washed by gentle pressure in repeated changes of saline solution in order to rid them of as much blood as possible. The small pieces were then ground with a mortar and pestle or with a Ten Brock grinder until the crushed tissues passed through a gauze filter several layers thick without leaving an appreciable residue. The ground cardiac tissue was then diluted to make a 10 per cent suspension and stored at 5 C. until used.

Antiserum was prepared in rabbits by giving over a period of one month intravenous injections of a 10 per cent suspension of the antigen in amounts beginning with 1 cc. and ending with 9 cc. Precipitin tests made according to the method recommended by Zinsser and Bayne-Jones [6] revealed an antibody content that produced a precipitate with the rat heart antigen in dilutions of 1:4,000. Control precipitin tests in which the immune rabbit serum and normal rat serum were used indicated the presence of variable amounts of antibody to rat blood serum as well as that to rat heart tissue.

Immediate Effects of an Injection of the Antiserum in Normal Rats.—Rat B9 (weight, 130 Gm.) was given 2 cc. of the antiserum, injected into the femoral vein. Respirations stopped in about thirty seconds, and the heart continued beating five minutes while artificial respiration was given. Three other rats, weighing between 100 and 250 Gm., were given 0.6, 0.5 and 0.3 cc. intravenously. They died between five and thirty minutes after the injection. The ones that lived a

5. Rich, A. R., and Gregory, J. E.: Bull. Johns Hopkins Hosp. (*a*) **72**:65, 1943; (*b*) **73**:239, 1943; (*c*) **75**:115, 1944; (*d*) **73**:465, 1943.

6. Zinsser, H., and Bayne-Jones, S.: A Textbook of Bacteriology, New York, D. Appleton-Century Company, Inc., 1939, p. 932.

few minutes had labored and gasping respirations, and a clear serous fluid dripped from their nostrils—about 3 cc. in 1 instance. (These effects were not observed in control experiments in which normal rabbit serum was used.)

Autopsy revealed in all rats conspicuous hyperemia, edema and hemorrhages of the lungs. A large amount of watery fluid was in the respiratory passages and there was an estimated 1 to 2 cc. in the pleural spaces. Histologic examination of the lungs revealed hyperemia, edema and hemorrhages. The alveolar walls were greatly thickened by widely dilated capillaries. The erythrocytes in some of the small vessels were without distinct cell boundaries and formed dense hyaline masses like thrombi. In the air spaces were finely granular precipitates and strands of fibrin. Some were obscured by hemorrhages. Sections of liver, heart and kidneys revealed only slight hyperemia.

Effects of Repeated Injections of the Antiserum.—Rats B10, B11 and B12 received repeated small injections of the antiserum intravenously, intramuscularly and intraperitoneally. Rat B10 received a total of 1.7 cc. over seven days, B11 7.5 cc. over fourteen days and B12 8 cc. over twenty days. After each injection there were labored respirations, an outpouring of nasal secretion and symptoms interpreted as abdominal distress. The animals writhed as if in extreme discomfort and assumed unnatural positions. Turned on their backs, they made little effort to right themselves. The effects varied somewhat with the route of administration, the writhing being more prominent after intraperitoneal injection.

Histologic examination of the lungs revealed a combination of retrogressive changes and a proliferative reparative process. The lungs of rat B11 had marked thickening of the alveolar walls by fibrin-like material and connective tissue cells with large vesicular nuclei. Between the cells were delicate and coarse irregular strands of fibrin and hyaline material. These changes were seen also about many of the small blood vessels. Scattered throughout the lungs were variable numbers of phagocytes, some containing carbon pigment and polymorphonuclear leukocytes. There were hyaline changes of the walls of some of the smaller blood vessels. About some were eosinophil leukocytes. In addition there were variable edema and hyperemia, depending on the time since the last injection of antiserum.

Rats receiving intraperitoneal injections had a striking proliferative response of the peritoneum, which was revealed on the surfaces of the liver and the bowel. The thickened capsule of the liver was formed mainly by fibroblasts with large vesicular nuclei, with edematous, faintly fibrillar and granular interstitial tissue that stained poorly. There was a scanty infiltration of small round cells.

Rats B13, B14 and B15 received intracardiac injections of antiserum. In each case the injection was continued as the needle was withdrawn in order to let some of the antiserum escape into the pericardial space. Autopsy revealed proliferative changes of the epicardium and the pleura, essentially the same as those of the peritoneum of the rats described in the foregoing paragraph. There were also focal regions of necrosis of the cardiac tissue in the path of the injecting needle. Muscle fibers showed hyaline degeneration and fragmentation. Frequently portions of the cytoplasm were removed, so that only cell outlines remained. Hemorrhages, fibrin deposits and variable infiltrations of polymorphonuclear

leukocytes were also present. Control animals that were given injections of normal rabbit serum had only poorly defined regions of myocardial edema.

Except in animals receiving intracardiac injections, there were no consistent lesions of the heart that resembled Aschoff bodies.

COMMENT

The antiserums obtained by using rat hearts as antigen when injected into rats intravenously in sufficient quantity (as little as 0.3 cc. with some serums) produced fatal reversed anaphylactic shock regularly. This anti-rat-heart serum contained precipitins for rat blood serum elements, as demonstrated, and probably to other tissue not organ specific. Therefore, the effects following injection of the serum cannot be ascribed to antibody for a given tissue element, such as connective tissue, endothelial tissue, myocardial muscle tissue or plasma proteins, but must be considered the combined effects of antibodies to the various tissue elements present in cardiac tissue. Anaphylactic shock in rats has been produced only with some difficulty [7] by the usual methods of sensitizing the animal with repeated inoculations of a protein and after a latent period giving a shock dose of the same protein. During this latent period circulating antibodies appear in the blood. When the shock inoculation is given, the antigen reacts with the antibodies, and anaphylactic shock results. In the experiments described here, however, the antibody was supplied artificially and reacted immediately with antigen which in this case is part of the normal tissues. The shock produced in either is similar. Both the Parkers [7b] and Pratt [7a] have described hyperemia, hemorrhages and edema of the lungs similar to the changes observed here. Congestion of the abdominal viscera, particularly of the intestine, also was noted.

Smadel [2] described a similar anaphylaxis-like reaction in rats with the administration of anti-rat-kidney serum. Histologic study of the tissue changes following these reactions was confined chiefly to the kidneys.

Since in most instances in the present study the antiserum was injected into the femoral vein, the first extensive capillary bed encountered was in the lungs. The antibody was probably "fixed" to the lung tissues and therefore was soon removed from the circulation. The resulting acute tissue reaction was analogous to the Arthus phenomenon with an antigen-antibody reaction causing extensive damage to the capillary walls, resulting in edema, hyperemia and hemorrhages. As suggested by Opie,[8] should antigen and antibody meet within endothelial cells and form a precipitate within the cytoplasm,

7. (*a*) Pratt, H. N.: J. Immunol. **29**:301, 1935. (*b*) Parker, J. T., and Parker, F., Jr.: J. M. Research **44**:263, 1923-1924.

8. Opie, E. L.: J. Immunol. **9**:259, 1924.

the injurious reaction would be especially severe, and the result would be expressed in increased permeability of the blood vessels. The serums used in this experiment were antiserums to normal rat tissue and therefore would produce an even greater reaction with tissue cells, such as endothelium, than would occur in the Arthus reaction produced in the usual way.

These experiments enabled a study of the effects of repeated antigen-antibody reactions involving given tissues. Usually anaphylactic shock can be produced only once in a given animal. Repeated reversed anaphylactic reactions were produced by injecting the antiserum into rats B10, B11 and B12. The end result in the lungs histologically was a marked proliferative and reparative change with fibrosis and conspicuous thickening of the alveolar septums with some perivascular infiltration and hyaline changes.

By injecting antigen directly into various viscera and body cavities of sensitized animals other workers [9] have produced essentially similar lesions.

There is a considerable similarity between the acute and the chronic pulmonary changes produced experimentally and the pulmonary changes seen in rheumatic fever. Epstein and Greenspan [10] described in the early stages of rheumatic pneumonia congestion of the alveolar capillaries with considerable fluid passing into the alveolar septums and the interlobar spaces and with large mononuclear cells being desquamated into the alveoli. Later, as the changes progress, there are thickening and mononuclear cell infiltration of the interlobar septums, with resultant fibrosis and organization. Rich and Gregory [5a] described similar pulmonary changes and pointed out the definite similarity between anaphylactic pneumonitis caused by drug or serum sensitivity and rheumatic pneumonitis. In the experiments described in this paper acute and chronic changes of the lungs of rats resembling the lesions described in human rheumatic pneumonitis were produced by reversed anaphylaxis.

Proliferative changes of serous cavities (peritoneal, pleural and pericardial) occurred in the cavities into which antiserums were injected. This apparently was the result of a local antigen-antibody reaction and was analogous to the reparative proliferative lesions of the lungs occurring with the repeated reversed anaphylactic shock. This proliferative response to an antigen-antibody reaction is characteristic of the Arthus phenomenon [9] and is further evidence of the potentials of immune reactions in the genesis of disease.

The focal lesions of the heart did not occur frequently enough to be ascribed to the effects of antiserums.

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9. Stenn, F.: Arch. Path. **26**:244, 1938.
10. Epstein, E. Z., and Greenspan, E. B.: Arch. Int. Med. **68**:1074, 1941.

SUMMARY

Anti-rat-heart rabbit serum was prepared by injecting a suspension of ground rat heart into rabbits. This antiserum when injected intravenously in sufficient quantities into rats produced an anaphylaxis-like shock, including hyperemia, hemorrhages and edema of the lungs, and death. Repeated inoculation of rats with small quantities of the antiserum produced proliferative and reparative changes of the lungs similar to the lesions described as rheumatic pneumonitis. When the anti-rat-heart rabbit serum was injected into serous cavities of rats, it produced marked proliferative changes.

Case Reports

DUODENITIS WITH DIVERTICULUM AND ECTOPIC PANCREATIC TISSUE

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IN THE case to be presented, the condition of the patient was diagnosed clinically as a deficiency state resulting from pathologic changes in the stomach and the duodenum. In considering the nature of these changes, the presumptive diagnosis was peptic ulcer probably undergoing cancerous changes and gradually leading to pyloric stenosis. Roentgenologic studies failed to confirm this possibility. Autopsy revealed an extremely severe form of duodenitis, sharply limited to the distal two thirds of the duodenum, with duodenal diverticula and ectopic pancreatic tissue in the proximal third.

Inflammation occurring in an anomalous organ is not unusual, but the location, the extent and the nature of the lesions in this case were such that it was considered worth reporting.

REPORT OF A CASE

H. T., a married woman 33 years of age, was admitted, Oct. 13, 1943, to the Hospitals of the American University of Beirut. She complained of epigastric pain and intermittent vomiting, from which she had suffered for the past eight years, accompanied for the last two months by ascites and edema of the lower extremities. Her social and past medical histories were essentially irrelative.

About eight years prior to admission she had an attack of severe diarrhea, with mucus, pus and a little blood in the stools accompanied with mild fever. The condition subsided in a month's time, but a few weeks later she began having attacks of mild epigastric pain and distention after meals, followed by nausea and relieved by vomiting. The epigastric distress began from fifteen to sixty minutes after meals, was never severe, had no relation to the type of food and was seldom relieved by alkalis. Occasionally she experienced hunger pains that were relieved by food. At the onset these attacks lasted several days, recurred once or twice a month and were worse during the colder seasons. However, she had no other complaints, and her general health remained fairly normal for five years.

Three years before admission she had daily spells of vomiting. The attack continued for a month then intermittently with progressive loss of weight. For the last three months she had amenorrhea and ascites with edema of the lower extremities. For the last two months she had a recurrence of diarrhea with pus and mucus and occasional tarry stools.

On admission the patient was afebrile but appeared seriously emaciated. In addition to the ascites and edema, she had a tender, reddish tongue. The heart sounds were weak, and the respirations were shallow but otherwise were normal. The white blood cell count was 7,000, with polymorphonuclears 86, lymphocytes 8 and large mononuclears 6 per cent. The red blood cell count was 4,220,000, the hemoglobin content, 10.9 Gm. per hundred cubic centimeters. There were no significant findings in routine and bacteriologic studies of the urine and the stool.

From the Department of Pathology, American University of Beirut.

Two days after her admission a fever developed that lasted for a week, her temperature fluctuating between 38 and 39 C. (100.4 and 102.2 F.) With proper medical care the vomiting and the diarrhea stopped; yet she presented dysphagia, refusing everything by mouth, and, in spite of feeding by vein, she lost ground rapidly and died twenty days after admission.

Laboratory Data.—Examination of the blood showed total plasma protein 2.5 mg. per hundred cubic centimeters of serum (albumin 1.0, globulin 1.5) and urea nitrogen 27 mg., sodium 292 mg. and chlorides 440 mg. per hundred cubic centimeters of blood. The test for sugar tolerance revealed a gradual rise from a fasting level of 60 mg. per hundred cubic centimeters of blood to 90 mg. within two hours, at which time a trace of sugar appeared in the urine. The Wassermann, Kahn and agglutination tests of the blood gave negative results. Analysis of the gastric juice showed no free acidity even after injection of histamine phosphate; a test for occult blood was positive; a test for lactic acid was negative. The ascitic fluid (3.5 liters obtained by paracentesis) had the characteristics of a transudate; bacterial culture produced no growth.

Report of Roentgenologist.—"The esophagus is free of defect. The stomach is moderately enlarged and contains a definite excess of mucus and secretion. Peristalsis and evacuation are sluggish. There is no demonstrable filling defect or niche suggestive of ulcer in the stomach. The duodenal cap is large and wide but shows no deformity or niche. The second portion of the duodenum is widened, and in both the second and the third portion there is stasis. The gastroduodenal curvature is of normal width. At six hours there is a slight gastric residue, but most of the opaque meal is seen in the lower small intestines and the cecum. At twenty-four hours the whole colon is outlined and shows no defect."

Autopsy.—The body was preserved in the refrigerator and was examined fourteen and a half hours after death.

The stomach was slightly enlarged and contained excess mucus. The mucosa of its body was thickened and mamillated. The pyloric orifice appeared normal.

The suprapapillary portion of the duodenum was dilated (circumference 16 cm. after formaldehyde fixation; its walls were thin, but the mucosal folds appeared normal. Three centimeters distal to the pylorus a patch of pancreatic tissue, 4.5 by 3 cm., was found incorporated in the anterior wall of the duodenum.

The head of the pancreas was firmly incorporated in the medial wall of the second portion of the duodenum. On cutting through it close to the papilla, a diverticulum was discovered, 8 mm. in length.

On the same level with the duodenal papilla, a nodule, 1.0 by 0.5 cm., was felt in the antimesenteric side of the wall.

The infrapapillary portion of the duodenum down to the jejunal junction was converted into a rigid tube by cicatricial connective tissue and adhesions involving the neighboring organs. These changes increased in intensity as the duodenojejunal junction was approached. The mucosal folds were not seen. The lumen was gradually narrowed, with corresponding thickening of the walls; where the duodenum was crossed by the root of the mesentery, the wall measured 7 mm. in thickness.

Eight centimeters distal to the level of the papilla, an indurated nodule, 1.5 cm. long by 1.0 cm. wide by 1.0 cm. deep, was seen protruding out from the antimesenteric side of the wall. It contained pocketed pus.

Apart from the cecum, which showed congestion, the rest of the intestines appeared normal. The glands of the mesenteric root were moderately enlarged. The pancreas was atrophic and weighed 20 Gm. only. The liver appeared fatty and contained in the left lobe a cavernous hemangioma, 2.0 cm. in diameter. The gall-

bladder was distended with half a liter of bile; its mucosa appeared normal. The biliary passages were patent. The adipose tissue was markedly depleted of its fat content. The blood was of a bright red color and was not clotted. There were no significant gross findings in the other organs.

Microscopic Examination.—(*a*) Stomach: The mucosa was infiltrated with plasma cells, lymphocytes and large mononuclears. The deeper portions of some of the glands showed cystic dilatation. In the pyloric region, however, the inflammatory changes were more pronounced, with partial destruction of glands and beginning fibrosis.

(*b*) Duodenum: The mucosa of the suprapapillary portion was infiltrated with round cells, but there was no destruction of epithelium.

The aberrant pancreatic tissue was of normal structure, though infiltrated with small round cells. It was separated from the mucosa by loose connective tissue containing a few of Brunner's glands; any trace of the muscle layer was lacking here. Externally it was covered by loose connective tissue containing small lymph glands and many dilated tortuous lymphatic vessels.

The diverticulum close to the papilla was made of mucosa and submucosa containing a few of Brunner's glands. There was a small islet of smooth muscle at its neck; otherwise no muscle layer could be seen. The lamina propria of this pouch was infiltrated with small round cells as was the rest of the mucosa of the suprapapillary portion. The pancreatic duct in this section was denuded of its epithelium.

The nodule at the level of the papilla was made up of densely packed small round cells and granulation tissue. It was covered by broken-down, degenerating muscularis mucosae—all that remained of the mucosa. The underlying muscle layer was partially eroded.

The infrapapillary portion of the duodenum showed extreme destruction of the mucosa. Epithelial structures were absent, and the wall was lined in different sections by thickened, cicatricial muscularis mucosae, by necrotic and granulating tissue or even by the fibrotic muscle layer of the duodenum. The submucosa and the serosa were extensively infiltrated with small round cells, with formation of lymph follicles. The muscle layer was infiltrated with these cells and showed marked destruction. The serosa was markedly edematous. All the layers showed cicatrizing granulation tissue.

The distal nodule was made up of a central focus of suppuration surrounded by inflammatory granulation tissue, which in turn was lined by hyalinizing cicatricial tissue. Few shreds of muscle were seen in its wall.

(*c*) Pancreas: This organ showed a mild chronic inflammation with cystic dilatation of some acini and patchy fibrosis.

(*d*) Other Organs: Small round cells were seen infiltrating the mucosa and the submucosa of the cecum. There was an old standing inflammation with fibrosis in the mesenteric lymph glands, although foci of polymorphonuclears were also seen. Multiple organizing thrombi were seen in the medium-sized veins of the lungs.

COMMENT

The two anomalies in this case were the diverticulum near the papilla and the ectopic pancreatic tissue.

Duodenal diverticula are not uncommon. Odgers,[1] quoting Grant and Linsmayer and giving the average of five other contributors, put the incidence in

1. Odgers, P. N. B.: Brit. J. Surg. **17**:592, 1930.

routine autopsies at 16, 3.3 and 2.5 per cent, respectively. Edwards [2] estimated the incidence as below 1 per cent. According to Illingworth,[3] Odgers [1] and others, duodenal diverticula usually remain symptomless. Odgers [1] expressed the opinion that duodenal diverticulitis is rare because of the relative sterility of duodenal contents and the comparatively large size and the dependent position of the diverticular opening into the bowel.

The intimate relation of primary duodenal diverticula to the pancreas or to ectopic pancreatic tissue is well known. In this case, the presence of ectopic pancreatic tissue in the wall of the distended proximal third of the duodenum together with the absence of the muscle layer was indicative of the existence of a shallow diverticulum there, which had been flattened out as a result of the dilatation of this portion. Moreover, as duodenal diverticula are often multiple (Odgers), the finding of another diverticulum incorporated in pancreatic tissue is in support of this view.

Since the epithelial covering of these two anomalies was intact, there is no ground for supposing that infection started in either one of them. But the peculiarity of the case is not so much the origin of the infection as the extent and the location of it. The infection may have originated in the pancreas and spread to the duodenum by way of the pancreatic duct. The atrophy of the pancreas, the destruction of the epithelium of the pancreatic duct and the infrapapillary location of the severe inflammatory changes of the duodenum would all lend support to this view. Or the infection may have started as primary gastritis or duodenitis. According to Kellogg and Kellogg,[4] Boyd [5] and Judd and Nagel,[6] chronic gastritis and chronic duodenitis are recognized clinical and pathologic entities, and frequently the two occur together. Whatever its origin, the infection once started probably became confined to one or more structural anomalies in the distal two thirds of the duodenum. The two nodules in the descending portion of the duodenum may well have been mucosal pouches with narrow outlets in which the infection either originated or became established, and from these pouches septic material was constantly washed out, causing severe and destructive inflammation of the surfaces with which it came in contact.

SUMMARY

After eight years of illness a 33 year old woman died with manifestations of nutritional deficiency. At autopsy the important lesions were confined to the distal two thirds of the duodenum and consisted of a granulating nodule at the level of the papilla and a suppurating nodule 8 cm. distal to it, as well as extreme destruction of the entire mucosa with extensive chronic inflammation and fibrosis of the wall. There was a diverticulum in the immediate vicinity of the duodenal papilla, and there was a patch of aberrant pancreatic tissue in the wall of the proximal third of the duodenum, where the muscle layer was absent. There was simple chronic inflammation of the entire stomach and of the proximal third of the duodenum. The pancreas was markedly atrophic.

2. Edwards, H. C.: Lancet **1**:169, 1934.

3. Illingworth, C. F. W.: Text-Book of Surgical Treatment, Edinburgh, E. & S. Livingstone, 1943, pp. 478 and 479.

4. Kellogg, E. L., and Kellogg, W. A.: Am. J. Surg. **21**:368, 1933.

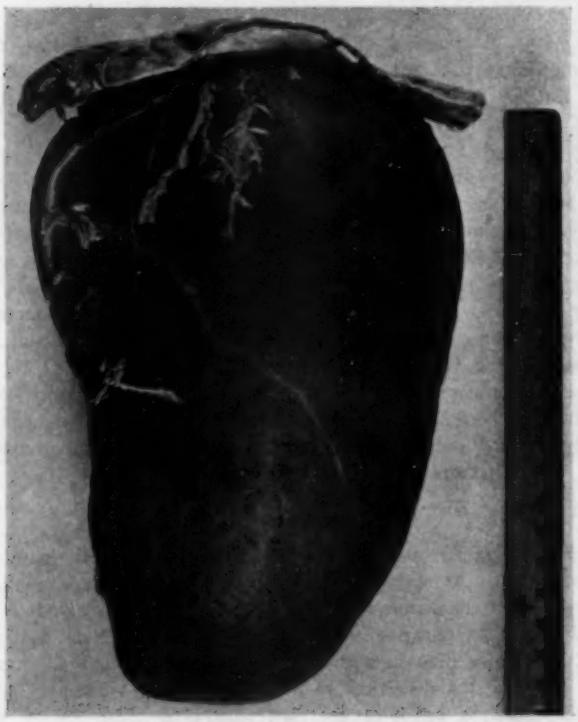
5. Boyd, W.: Text-Book of Pathology, ed. 4, Philadelphia, Lea & Febiger, 1943, p. 566.

6. Judd, E. R., and Nagel, G. W.: Ann. Surg. **85**:380, 1927.

COMPLETE ABSENCE OF THE LEFT LOBE OF THE LIVER

GEORGE G. MERRILL, M.D., BALTIMORE

COMPLETE absence of the left lobe of the liver is an extremely rare congenital anomaly. Only 1 case of it is described in the literature.[1] The other reported cases of anomalies of the lobes of the liver were cases of anomalies of shape or of increased number of lobes, rather than of a decreased number. In the single previously reported



Anterior surface of the liver, showing complete absence of the left lobe.

case of absence of a lobe there was a right lobe of normal size and shape, with normal bile ducts, normal blood vessels and normal microscopic appearance. There was no compensatory increase in the size of the lobe, in contrast to the case now described. The embryologic defect must occur at an extremely early stage of development, as

From the Johns Hopkins Hospital.

1. Messing, A., and Montague, M. F.: Anat. Rec. **53**:169, 1932.

the anlage of the right and that of the left lobe of the liver normally appear in the first few weeks of embryonic life, being seen in the 3 mm. embryo.

REPORT OF A CASE

The present anomaly is the only one of the kind seen in some 19,000 autopsies at the Johns Hopkins Hospital. It occurred in a 36 year old white woman who died of carcinoma of the right bronchus, which had metastasized to lymph nodes, the lungs, the ovaries, the right adrenal medulla and vertebrae. There were no metastases in the liver. Clinically the case was interesting because of the uncertainty regarding the primary origin of the tumor. The fact that the elongated right lobe of the liver extended downward so that its lower edge was palpable in the right lower quadrant of the abdomen led most examiners to regard this palpable abdominal mass as the primary tumor rather than as an unusually shaped normal liver.

The most striking features of the liver at autopsy were the absence of the left lobe and the elongation of the right lobe. There was no evidence of any left lobe ever having been present. The right lobe was greatly elongated, with its lower margin reaching down well into the right lower quadrant of the abdomen. It measured 22 cm. in length, 13.5 cm. in width and 7 cm. in thickness in the upper third. The circumference at the point of maximal thickness in the upper third was 33.5 cm. The anterior surface was smooth. The superior surface and the ligaments were normal except for the absence of the left lobe. The caudate lobe was normal. The posterior surface of the liver had shallow impressions marking its relationships to the stomach, the duodenum, the colon and the right kidney. The gallbladder lay in a transverse position, with its fundus at the median border of the liver 10 cm. above the lower margin of the right lobe. The neck of the gallbladder was 7 cm. lateral to the fundus, and the cystic and common bile ducts extended laterally and slightly downward to reach the duodenum at about the same level as the lower margin of the fundus.

Histologically, the liver was entirely normal. There was no evidence of altered function of the organ either clinically or pathologically. No other congenital anomalies were found. No vascular abnormalities were apparent.

Obituaries

SIMON FLEXNER, M.D.

1863-1946

Simon Flexner remained for a time the last survivor of the group of physicians who acquired professional maturity during the early period of the Johns Hopkins Hospital when Welch, Osler, Halsted and Kelly collected about themselves younger men of conspicuous ability and transmitted to them their enthusiasm for investigation in medicine. The close association of Dr. Flexner and Dr. Welch is described in the biography of Welch by Flexner and his son.[1] They designate this early period "The Heroic Age of American Medicine." Dr. Flexner's greatest contribution to American medical science was the organization and administration of the Rockefeller Institute for Medical Research, but the background of this accomplishment was his own development as pathologist and investigator.

Abraham Flexner, the distinguished brother of Simon, has told in his memoirs the history of their family. His father came to America from Germany in 1853 and his mother two years later. There were seven boys and two girls, and their father was eager that his sons should be doctors, lawyers and scholars. He died when Simon was 19 years old, but his wish was amply fulfilled. Abraham Flexner has given a moving account of the steadfast courage with which his mother maintained her numerous family under adverse conditions.

Simon Flexner, who became Doctor of Science of Yale, Harvard and Princeton, Doctor of Laws of Hopkins and Cambridge and Fellow of Oxford and received honorary degrees from at least ten other universities, held no collegiate degree. He received, then 26 years old, the degree of Doctor of Medicine from the Louisville Medical School in 1889. This school had had as transient teachers, Daniel Drake, Samuel Gross, Austin Flint and Elisha Bartlett and, Dr. Flexner said in "A Half Century of American Medicine," in his day still bore the impress of these teachers.[2] Nevertheless, lectures followed one another in bewildering sequence during the long days of four winter months and were repeated precisely during a second year, at the end of which graduation with the degree of Doctor of Medicine was all but automatic. Of laboratory instruction there was none, and the dissecting room provided the one place where instruction might be regarded as practical.

1. Flexner, S., and Flexner, J. T.: William Henry Welch and the Heroic Age of American Medicine, New York, Viking Press, Inc., 1941.

2. Flexner, S.: Science **8**:505, 1937.

Chance, in accord with the usual meaning of the word, seems to have had an insignificant part in the development of Dr. Flexner's career. Even before he left Louisville the urge to pursue a career in medical science was evident and he had published in local medical journals three papers in which he discussed the relation of some aspects of pathologic histology to clinical medicine. In one of these he describes Ehrlich's observations on the structure of the blood corpuscles, and in another he reviews several of Löffler's publications concerning the diphtheria bacillus and discusses its diagnostic significance.

"That fall" (1891), he says, "I came to Baltimore eager to work in Welch's laboratory. I had not attempted to practice, but had used Delafield's and Prudden's book,[3] as well as other simple texts to teach myself a little pathology. My preparation was the most rudimentary". Dr. Welch admitted the applicant to his course for graduates in medicine, though he did not seem particularly interested in the young man's desire to study pathology. Flexner spent all of his time in the pathologic laboratory and was able to complete a study which Dr. Welch regarded as worthy of publication. Dr. Welch's attitude toward him, he says, changed immediately, but this interest took a form that dismayed him. When he asked for permission to take Dr. Welch's course in bacteriology, Dr. Welch advised him not to take it. "There is no occasion to take the course. Study a problem." He followed this advice and abandoned his plan to return to Louisville and, as he says, eke out a living from pathology. Very soon (1892) he was appointed Fellow in Pathology and became, as he terms it, a sort of understudy to Councilman.

As Fellow in Pathology Dr. Flexner, in association with Dr. Welch, made a study of the histologic changes produced when animals were inoculated with the diphtheria bacillus and of the focal destruction of cells caused by soluble products of the micro-organism. When Councilman left the laboratory in 1892, Flexner was appointed as his successor. The industry with which Flexner developed his knowledge in the fields of pathology and bacteriology is well shown by the reports of the meetings of the Medical Society of the Johns Hopkins Hospital. Here doubtless the reputation of the younger members of the staff of the hospital was established. He presented many case reports of anatomic lesions, especially of those of tuberculosis and typhoid, bacteriologic observations (Bacillus pyogenes filiformis, nov. spec.), and notes on amebic abscess of the jaw, together with more elaborate studies in which the medical literature related to the subject was fully discussed. The routine bacteriologic examinations which accompanied

3. Delafield, F., and Prudden, T. M.: A Handbook of Pathological Anatomy and Histology, New York, William Wood & Company, 1885.

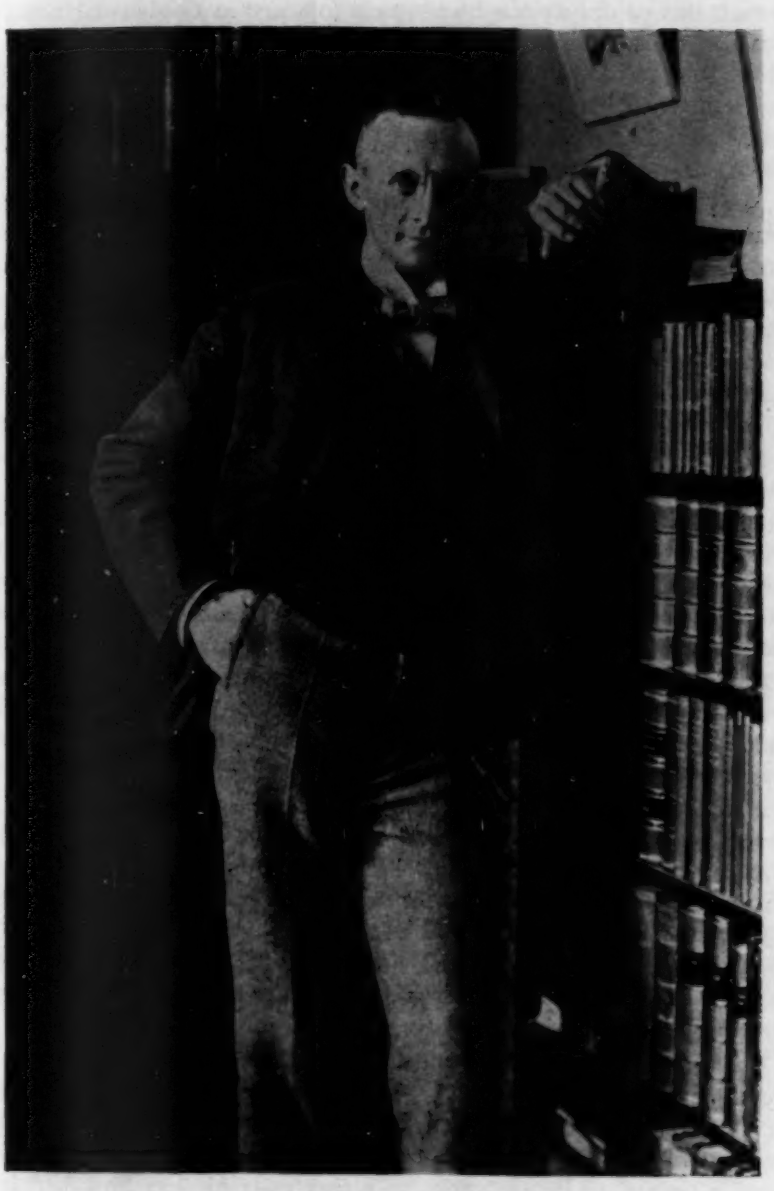
autopsies were a significant factor in building up his comprehensive knowledge of the etiology and the pathology of disease. The detailed studies of terminal infections and of the etiologic factors of acute peritonitis which he published show with what meticulous care these bacteriologic observations were made.

A monograph on the pathologic changes caused by toxic protein substances ("The Pathology of Toxalbumin Intoxication") was published,[4] in which emphasis was placed on the focal character of the lesions that were produced in parenchymatous organs especially the liver and lymphoid tissue, by the soluble toxin of the diphtheria bacillus and expressed the view that the lesions of human and of experimental diphtheria were identical. They were compared with the localized destruction of tissue produced by the poisons abrin and ricin, from the seeds of the castor oil plant. These substances, Ehrlich had shown, were, like diphtheria toxin, capable of producing by immunization antitoxins that inhibited their injurious action. As an example of toxic proteins derived from the animal kingdom Flexner used the blood serum of one animal introduced into another of a different species. In addition to destruction of blood corpuscles and resulting hemoglobinuria he found localized death of cells in lymphoid tissues and in parenchymatous organs, followed in the latter by evidence of chronic change. It is noteworthy that, as a by-product of his study of toxalbumin intoxication, he described several experiments which forecast anaphylaxis nearly a decade before it was recognized by Richet. He said: "Animals that had withstood one dose of dog serum would succumb to a second dose given after the lapse of some days or weeks, even when the dose was sublethal for a control animal." As Morgenroth has pointed out, Magendie, using egg white, made a similar observation.

During six years Dr. Flexner lived in the Johns Hopkins Hospital as one of the resident staff and, like his associates of the period, including Dr. Osler, remained in residence after he had become one of the senior members of the medical faculty. The accompanying portrait of him is reproduced from a photograph made at that time. In 1893 he was abroad and studied pathology in Prague and in Strasbourg, for a time as a student of von Recklinghausen. During the summer months several years later, working in the laboratory of Jacques Loeb in the Marine Biological Laboratory at Woods Hole, he studied the regeneration of the nervous system of planarians and the structure of anomalous forms produced experimentally.

Dr. Welch had a keen interest in pathologic anatomy and a wide knowledge of the subject, but he was primarily concerned with the etiology and the pathogenesis of disease. In the universities of the

4. Flexner, S.: Bull. Johns Hopkins Hosp. 6:259 1897.



SIMON FLEXNER, M.D.
1863-1946

United States general pathology and experimental pathology have not been separated as organized departments from pathologic anatomy, though this procedure has been widely followed in Continental Europe. Pathology has included the functional as well as the anatomic aspects of the subject. When Dr. Flexner was promoted to a full professorship before he left the Johns Hopkins University he received what may have seemed to him the paradoxic title of "professor of pathological anatomy."

Two events significant in reference to Dr. Flexner's subsequent career occurred before he left Baltimore. He was designated by the State Board of Health of Maryland to investigate an epidemic of cerebrospinal meningitis that occurred in mining towns in the mountainous district of Western Maryland. Information about 120 cases was collected, and two autopsies were performed. It is noteworthy that "Micrococcus lanceolatus," already found in association with lobar pneumonia, was at this time regarded as the probable cause of cerebrospinal meningitis, and in exudate obtained from the meninges Flexner found diplococci within and about phagocytic cells.

In the period immediately following the war with Spain, at a time when the United States Army was engaged in suppressing the Filipino revolt, a commission consisting of Dr. Flexner and Dr. Lewellys Barker was appointed under the auspices of the Johns Hopkins University to make a study of the diseases of the Philippine Islands. J. M. Flint, T. P. Gay and John W. Garrett accompanied the commission as voluntary workers. A report on the diseases prevailing in the neighborhood of Manila was made, but the most important result of the undertaking concerned the cause of bacillary dysentery. Strains of the bacillus of dysentery brought back from the Philippine Islands were the means by which, as the result of investigation by many observers, it became evident that the disease was caused by two forms of the micro-organism, since known as the "Shiga" and "Flexner" types. Later, at the Rockefeller Institute, with Sweet he showed that the soluble toxin obtained from the Shiga type of the dysentery bacillus when introduced into the circulating blood of rabbits reproduced the characteristic intestinal lesions of human dysentery.

The almost immediate influence of Welch's laboratory on the development of pathology in this country is shown by the appointment of Dr. Councilman to the professorship of pathology at Harvard University and a little later by that of Dr. Flexner to the professorship at the University of Pennsylvania. Flexner assembled in Philadelphia a group of able young assistants, including Pearce, Bunting, Yates and Noguchi. The latter came unexpectedly as the result of a casual meeting in Japan preceding Flexner's visit to the Philippines. The activity of this laboratory during three years is indeed astonishing.

Flexner continued his studies of toxalbumins and the reactions of the immunized animal to them. He directed Noguchi to a highly successful study of the nature and the mode of action of snake venoms. He made experimental studies of pancreatic disease, begun in Baltimore and later continued in New York. He demonstrated a fat-splitting enzyme in foci of fat necrosis. He undertook experiments to define the conditions under which hemorrhagic necrosis of the pancreas is produced, and he showed later that the necrosis which develops when bile is introduced into the pancreatic ducts is referable to the bile salts. He described hitherto unrecognized thrombi produced by agglutination of red blood corpuscles. He planned to write a textbook of pathology and had begun the preparation of the illustrations for it. Had he remained in Philadelphia, he would doubtless have fulfilled this purpose.

Dr. Flexner supervised the organization of the Ayer Clinical Laboratory of the Pennsylvania Hospital and was its first director. Longcope as resident pathologist was the first of a succession of able pathologists who while associated with this laboratory made significant contributions to pathology and clinical medicine.

Simon Flexner, in 1903, married Helen Whitall Thomas, a member of a family of Baltimore intimately associated through her father and her sister with the development of Johns Hopkins University and Bryn Mawr College. As an author she has upheld the scholarly tradition of her family. Their children are William Welch Flexner, professor of mathematics, and James Carey Thomas Flexner, an author who collaborated with his father in writing the biography of Dr. Welch.

Mr. John D. Rockefeller, in 1901, gave a fund to promote research in medicine, to be spent within a period of ten years. The Rockefeller Institute for Medical Research was established under the guidance of an advisory board, which ultimately became the board of scientific directors. Of this board Dr. Flexner became a member. During two years the fund was expended in small grants in aid of medical research. In the second year Mr. John D. Rockefeller Jr. informed the board that his father had assigned to the institute a much larger sum, again to be spent within a subsequent period of ten years. Part of this fund was designated for the purchase of land and the erection of a laboratory building in New York. Dr. Flexner accepted the directorship of the institute. He studied physiologic chemistry for a time in the laboratories of Emil Fischer and of Salkowski in Berlin and then, pending completion of the new laboratory, began work with a small staff in two houses temporarily converted into laboratories. Significant events in the development of the Rockefeller Institute for Medical Research were the opening of the original laboratory building in 1906, the establishment of a hospital for clinical investigation, the inaugu-

ration of a laboratory of animal pathology at Princeton and the establishment of a department of plant pathology at the same place. The institute in New York increased greatly in size and scope.

The dominant purpose of Dr. Flexner's later career was the success of the institute as a center of fundamental and applied research, broadly included in the domain of the medical sciences. He personally sought out and pursued fruitful problems for investigation and by his own example pointed the way to much of the later work of the institute in pathology and microbiology.

The prompt and widespread recognition which the Rockefeller Institute for Medical Research received was in no inconsiderable part the result of Dr. Flexner's studies concerning the control of epidemic cerebrospinal meningitis. His attention had been directed to this subject while he was still in Baltimore, but epidemics of the disease that occurred in New York in the winter of 1904 and the spring of 1905 gave the problem immediate urgency. Approximately 3 of 4 of those who were attacked died. He was soon able to confirm the observation that the disease could be reproduced in monkeys by bringing the meningococci into contact with the meninges. The changes produced resembled those that occurred in man, though the quantity of meningococci necessary to cause death was considerable and there was, he believed, scant multiplication. The experimental disease of the monkey afforded favorable opportunity to determine the value of serum obtained by immunization of the horse. Monkeys were protected when the serum was directly introduced into the subdural space by spinal puncture. Although this procedure had been employed in the treatment of the disease, its value was uncertain. With Jobling, Dr. Flexner published the results of intraspinal injections of the serum in 47 patients with meningitis, of whom only 12 died. On the basis of these observations they advised a wider trial of the antiserum. Later they found convincing evidence of its value and recorded the results of the treatment of 393 patients, of whom three fourths recovered. If treatment was begun early in the disease, the results were more favorable; of those who received subdural injections from the first to the third day of its course, only 16.5 per cent died. An analysis of the data concerning 1,300 patients who were carefully observed during treatment was published five years later, and the results were essentially the same as those previously recorded.

Studies of the transplantation of tumors were published by Flexner, Jobling and Menten in the first of a series of monographs issued by the Rockefeller Institute. The current studies of the period were in great part concerned with the conditions under which tumors of mice could be transmitted from one animal to another. The tumor of the rat studied by Flexner and Jobling was regarded by them

as an embryoma which at first had a simple carcinomatous structure but which with repeated transplantation assumed the characteristics of adenocarcinoma. Known as the Flexner-Jobling tumor, it has been perpetuated in laboratories in many parts of the world and has been the object of much experimentation.

Dr. Flexner published in 1910 the first of a series of studies of poliomyelitis, and these were the earliest of many important studies of virus diseases made at the Rockefeller Institute. They began like the studies of epidemic meningitis with the transmission of the disease to monkeys. Landsteiner and his associates had shown that poliomyelitis could be transmitted to these animals by intraperitoneal and intracerebral inoculation and that the agent was conveyed by material that had passed through a Berkefeld filter. Flexner and Lewis, a few months later, confirmed these observations. Dr. Flexner's interest in poliomyelitis centered about its pathogenesis, with especial reference to the mode of the body's elimination of the infectious agent, the manner in which the agent made its entrance into the normal host and the route by which it reached the meninges and the central nervous system. The virus was demonstrable only by the inoculation of monkeys with the production of the disease, and answers to any questions concerning it were dependent on this laborious procedure. He and his co-workers, Clark and Amoss, demonstrated that the disease of monkeys was almost identical with that of human beings. Gastrointestinal administration of infected material was seldom followed by the disease, but animals were readily infected if the same material was applied to the mucosa of the nose. Flexner and Amoss described experiments which indicated that the virus passed along the olfactory nerve to the olfactory bulb and thence to other parts of the central nervous system.

Poliomyelitis was produced when infected material was inoculated into any part of the nervous system, but it seldom occurred when infected material was introduced into the circulating blood. The meninges and the choroid plexus formed a barrier between blood and cerebral substance, but this barrier could be overcome with mildly irritant aseptic fluids, such as normal monkey or horse serum or isotonic solution of sodium chloride, which when brought into contact with the meninges by intraspinal injection inflamed or even slightly altered the integrity of the meninges and the choroid plexus.

Dr. Flexner seems to have assumed that the conditions that had favored his own research would promote that of others. He has outlined his conception of the administration of research. "This meant in practice choosing promising young men, affording them suitable opportunities for work under little or no direction and retaining over a term of years those who showed the greatest aptitudes in finding

themselves and dealing successfully with the problems on which they were engaged." "Only an exceptional person, of course, is gifted with the power to extend knowledge, but a much larger number of persons can, under direction, add to the sum total; both kinds of ability were utilized by the growing institution. The power of the more gifted was enlarged through the use of the less, and the incidental training secured by the latter became a valuable asset in the educational expansion of the country."

Dr. Flexner's wide knowledge of medical science with especial reference to public health and his demonstrated ability to solve problems requiring investigation established confidence in the soundness of his judgment. His advice was often sought by educational institutions that were contemplating changes in their organization. He was an earnest advocate of the "full time" plan of clinical teaching. Medical schools asked for his aid when they were attempting to find the best available persons to fill vacant professorships. When an epidemic of plague threatened the Pacific Coast, he was appointed by the national government a member of a commission to find means for its control. He was a member of the State Council of Health of New York, and for many years its chairman.

He served during a period of years as a member of the board of trustees of the Rockefeller Foundation and also of that of the Carnegie Institute of Washington. Shortly after his retirement from the directorship of the Rockefeller Institute of Medical Research he was appointed Fellow of Balliol College of Oxford and Eastman visiting professor. In his later years he renewed his association with the Johns Hopkins University as a member of its board of trustees.

Dr. Flexner's career includes a period which begins with the return of medicine of the United States to the current of European science and ends with a time in which research has become a dominant feature of the medicine of this country. He has had an outstanding part in this development.

EUGENE L. OPIE

Notes and News

Appointments, Etc.—Fritz Levy, formerly pathologist to the Davis Memorial Hospital, Elkins, W. Va., is now pathologist to the Veterans Administration Facility, Huntington, W. Va.

R. A. Moore, professor of pathology, Washington University School of Medicine, St. Louis, has been appointed acting dean.

Kenneth Goodner, of the International Health Division of the Rockefeller Foundation, has been appointed professor of bacteriology and immunology at Jefferson Medical College, Philadelphia.

At the Medical College of Virginia, Richmond, George Z. Williams has been promoted to professor of pathology; in the department of bacteriology and parasitology R. L. Thompson has been appointed associate professor and William A. Summers assistant professor.

Commander H. M. Zimmerman (MC), associate professor of pathology at Yale University and recently executive officer of the United States Naval Medical Reserve in the Pacific area, has been appointed chief of the laboratory division of Montefiore Hospital, New York.

Death.—Ernst Freund, biochemist, founder of the first hospital clinical laboratory in Austria, originator of the Freund-Kaminer test for cancer, died in London June 2, 1946, at the age of 82.

Award.—The Alvarenga Prize has been awarded to William H. Feldman, of the Mayo Foundation, in recognition of his studies on the chemotherapy of tuberculosis.

Society News.—The 1947 meeting of the Society of American Bacteriologists will be held in Philadelphia during the week beginning May 11, under the presidency of James Craigie, of the University of Toronto. The secretary-treasurer is L. W. Parr, George Washington University, Washington, D. C.

Institute of Pathology.—The Rhode Island Hospital, Providence, has established an institute for clinicopathologic service to other hospitals in the state.

Needs of War Devastated Libraries.—Medical and scientific books of the last decade and periodicals are greatly needed. Shipments should be sent prepaid to American Book Center, care of the Library of Congress, Washington 25, D. C. The center depends on gifts from individual donors and institutions.

The National Institute of Health.—Applications for grants-in-aid from investigators in the field of pathology will be considered. Applications should be addressed to the Chief, Research Grants-in-Aid Office, National Institute of Health, Bethesda 14, Md.

Since the announcement, June 3, 1946, of the publication of a journal to be called *Research in Medical Science,* unforeseen circumstances have interrupted the program. While publication has had to be abandoned for the present, it is hoped that the National Institute of Health will be able to carry out the original plan at a later date.

A pathologic study section has been formed under the research grants-in-aid program of the National Institute of Health. The basic purpose of this section is to foster research in general pathology. The section consists of Paul R. Cannon, as chairman, W. A. DeMonbreun, W. D. Forbus, Harry Goldblatt, J. S. McCartney, A. R. Moritz, Arnold Rich, J. F. Rinehart and H. P. Smith as members, and R. D. Lillie, National Institute of Health, as secretary. The section met Aug. 16, 1946, in Bethesda.

Books Received

DISEASES OF THE RETINA. By Herman Elwyn, M.D., senior assistant surgeon, New York Eye and Ear Infirmary. Pp. 583, with 170 illustrations, 19 in color. Price $10. Philadelphia and Toronto: The Blakiston Company, 1946.

The retinal diseases are considered under eight headings: diseases from disturbances of circulation; diseases from vascular malformations; hereditary degenerations; inflammatory diseases; tumors; retinal detachment; developmental anomalies; radiation injuries. The descriptions and discussions are marked by careful competence. The writing is clear and concise. There are many short, well chosen bibliographies. The illustrations, selected from many sources, are uniformly excellent. It is an attractive book that meets well the present special and general need of a complete and systematic presentation of the diseases of the retina.

HUMAN TORULOSIS: A CLINICAL, PATHOLOGICAL AND MICROBIOLOGICAL STUDY WITH A REPORT OF THIRTEEN CASES. By Leonard B. Cox, M.D. (Melb.), M.R.C.P. (Edin.), F.R.A.C.P., and Jean C. Tolhurst, M.Sc. (Melb.). Pp. 149, with 67 illustrations. Price, 25 shillings Melbourne: Melbourne University Press, 1946.

Thirteen new Australian cases of torulosis of the nervous system are reported in detail with illustrations. All phases of torulosis, spontaneous as well as experimental, are considered on the basis of the cases studied and of a thorough review of the literature in question. There is a comprehensive bibliography. The monograph is a valuable contribution to the study of torulosis.

THE JOHN AND MARY MARKLE FOUNDATION. Annual Report, 1945. Pp. 135. New York (14 Wall Street), 1946.

MEDICAL JURISPRUDENCE AND TOXICOLOGY. By John Glaister, J. P., D.Sc., M.D., F.R.S.(Edin.), of the Inner Temple, barrister-at-law, etc., Regius professor of forensic medicine, University of Glasgow; formerly professor of forensic medicine, University of Egypt, Cairo, and medicolegal consultant to the Egyptian Government. Eighth edition. Pp. 691, with 222 illustrations, 89 in color. Price $8. Baltimore: Williams & Wilkins Company, 1945.

The impression left with the reader of this eighth edition of a well known text is that contrary to a statement in the "Preface," it has not been "fully revised." Through the years the definition and clarity of the photographs have progressively deteriorated in the several editions to a point where the captions are now sometimes essential for the identification of the object portrayed.

Much remains to be desired concerning the presentation of the pathology of trauma. The illustrations are of scant assistance to the descriptions of gross anatomic findings, and histopathology is generally neglected. Physiologic mechanisms are either ignored or inadequately elucidated. It is questionable whether "cardiac failure due to inhibition induced by swimming while the progress of digestion was in active operation" can be a satisfactory certification of the cause of death.

No information is included concerning the methods of identifying metallic and powder residues in and near wounds in the investigation of deaths caused by gunfire. No mention is made of the effect of hydrogen ion concentration on the preservation of spermatozoa in cases of rape. The chemistry of the blood of the right and that of the left side of the heart is not discussed in relation to the diagnosis of death by drowning.

The section on toxicology, nearly a third of the text, is a compendium of brief clinical summaries and descriptions of chemical tests for various toxic agents, without discussion of pathologic or biochemical mechanisms. Acetylsalicylic acid poisoning, a primary respiratory alkalosis, most certainly will not be cured by the administration of alkali.

The illustrative cases of crimes make entertaining reading, but it is doubtful whether the intricacies of British and Scottish law are of value to American readers.